

Health and Environmental Effects
Profile for 2,4-Toluenediamine

(U.S.) Syracuse Research Corp., Cincinnati, OH

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE
FOR 2,4-TOLUENEDIAMINE

ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
OFFICE OF HEALTH AND ENVIRONMENTAL ASSESSMENT
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16. ABSTRACT

The Health and Environmental Effects Profile for 2,4-Toluenediamine was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. 2,4-Toluenediamine has been evaluated as a carcinogen. The human carcinogen potency factor (q₁*) for 2,4-toluenediamine is 3.2 (mg/kg/day)⁻¹ for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. The RQ value for 2,4-toluenediamine is 100.

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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEP's are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1985.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic exposures. An RfD is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, RfDs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEP's will become part of the EPA RCRA and CERCLA dockets.

ACKNOWLEDGEMENTS

The initial draft of this report was prepared by Syracuse Research Corporation under Contract No. 68-03-3228 for U.S. EPA's Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH. Dr. Christopher DeRosa was the Technical Project Monitor and Helen Ball was the Project Officer. The final documents in this series were prepared for the Office of Solid Waste, Washington, DC.

Scientists from the following U.S. EPA offices provided review comments for this document series:

Environmental Criteria and Assessment Office, Cincinnati, OH
Carcinogen Assessment Group
Exposure Assessment Group
Reproductive Effects Assessment Group

Editorial review for the document series was provided by:

Judith Olsen and Erma Durden
Environmental Criteria and Assessment Office, Cincinnati, OH

Technical support services for this document series were provided by:

Bette Zwayer, Pat Daunt, Karen Mann, and Jacky Bohanon
Environmental Criteria and Assessment Office, Cincinnati, OH.

EXECUTIVE SUMMARY

2,4-Toluenediamine undergoes reactions typical of amines, including salt formation, alkylation, amide formation (Morrison and Boyd, 1973), acylation, isocyanide formation, di- and tetra-azotization, oxidation by neutral and basic permanganate and hydrolysis in the presence of ammonium bisulfate yielding methylresorcinol (OHM/TADS, 1985). In 1984, 186.7 million pounds of 2,4-toluenediamine were produced (USITC, 1985) and in 1983, U.S. imports were 226,060 pounds (USITC, 1984a). 2,4-Toluenediamine is produced by the catalytic hydrogenation of 2,4-dinitrotoluene using a variety of temperatures, pressures and solvents, and supported noble metal catalysts, or Raney or supported nickel catalysts. Continuous, stirred flow, liquid-phase reactors are used in most commercial processes (Milligan and Gilbert, 1978). 2,4-Toluenediamine is usually produced as a mixture of 2,4- (80%) and 2,6-toluenediamine (20%), with smaller quantities produced as a 65/35 mixture of 2,4- and 2,6-toluenediamine (OHM/TADS, 1985). 2,4-Toluenediamine is used primarily in the production of toluene diisocyanate (Milligan and Gilbert, 1978; Hawley, 1981), in the production of dyes (Hawley, 1981) and in the preparation of impact-resistant resins, polyimides with superior wire-coating properties, benzimidazolethiols (antioxidants), hydraulic fluids, urethane foams, fungicide stabilizers and sensitizers for explosives (Milligan and Gilbert, 1978).

According to Lyman et al. (1982), 2,4-toluenediamine is not expected to hydrolyze significantly. Based on an estimated Henry's Law constant of 1.2×10^{-9} atm·m³/mol (Lyman et al., 1982), 2,4-toluenediamine will not volatilize from water. The very low estimated vapor pressure of 2,4-toluenediamine at 20°C and its large estimated water solubility suggest that

little or no volatilization from soil will occur. Aqueous photooxidation (Mabey et al., 1981) and aerobic biodegradation (Matsui et al., 1975) are expected to be the major factors in the environmental fate of 2,4-toluenediamine. Atmospheric and aqueous photolysis may occur, given the absorption maximum of 294 nm ($\epsilon = 2891$ L/M cm) (Sadtler, 1960). 2,4-Toluenediamine has an estimated $\log K_{oc}$ value of 1.56 (Lyman et al., 1982); it is not expected to bind tightly to soil (Kenaga, 1980) and should leach to groundwater. This conclusion, however, is based solely on physical interactions. Aromatic amines are known to form covalent bonds to humic materials (quinone-like compounds) followed by slow oxidation (Parris, 1980). Although no such data were available, 2,4-toluenediamine may also react in this way and will most likely remain in the soil much longer than the purely physical interactions suggest. According to Veith et al. (1979), an unspecified isomer of toluediamine exhibits a BCF of 91, and the estimated BCF for 2,4-toluenediamine is 1.06 (Lyman et al., 1982). These BCF values indicate that bioconcentration will not be significant.

The amount of 2,4-toluenediamine released to water as a result of its production in 1977 was estimated to be 13.6 million pounds (Johnson et al., 1980). 2,4-Toluenediamine was found to leach into water from boil-in-bags and retortable pouches, achieving average concentrations of 0.25 and 2.875 ppb, respectively (Snyder and Breder, 1982). The amount of 2,4-toluenediamine released to the air as a result of its production in 1977 was estimated to be 1.54 million pounds (Johnson et al., 1980). Additional monitoring data are required before daily intake values for the general population from exposure to water, air and food can be estimated. The concentration of 2,4-toluenediamine in air samples taken during the production of polyurethane-coated wire ranged from ND (<0.0005) to 8.13 mg/m³,

with an average concentration of 0.99 mg/m³. The highest concentration observed was detected in the exhaust ventilation (Rosenberg, 1984). Air concentrations in samples of the working atmosphere of the Olin Chemical Company, a producer of 2,4-toluenediamine, ranged from 0.008-0.39 mg/m³ in one survey and from ND to 0.038 mg/m³ in a second survey (Ahrenholz, 1980).

Absorption of 2,4-toluenediamine was ~90% after oral administration to rats (Sauerhoff et al., 1977) and ~54 and ~24% after a 24-hour dermal application to monkeys and humans, respectively (Marzulli and Green, 1978; Marzulli et al., 1981). Absorption by inhalation has not been evaluated. After oral and intraperitoneal administration, the liver and kidneys are among the organs with the highest concentration of 2,4-toluenediamine in rats and mice, while the testes are among the organs with the lowest concentration (Unger et al., 1980; Allied Corp., 1982; Grantham et al., 1979; Du Pont, 1974). The half-life of 2,4-toluenediamine is different in different organs, being shortest in the kidney (9.1 hours) and longest in the skin (29.2 hours) (Unger et al., 1980). Metabolism of 2,4-toluenediamine may occur by hydroxylation of ring carbons, acetylation of amino groups and oxidation of the methyl group depending upon species and strain (Waring and Pheasant, 1976; Grantham et al., 1979). Although Du Pont (1974) reported that the rate of elimination of 2,4-toluenediamine in rats approached first-order kinetics, Sauerhoff et al. (1977) reported biphasic elimination in rats and Unger et al. (1980) report biphasic elimination in mice. Elimination of a single oral dose (77 mg/kg) of 2,4-toluenediamine is virtually complete in mice in 2 days, but is not complete in rats until 6 days after dosing (Grantham et al., 1979). The increased clearance of 2,4-toluenediamine in mice as compared with rats may be due to increased conjugation of 2,4-toluenediamine metabolites to glucuronic acid, increased oxidation rates

to form benzoic acid, which can be conjugated to glycine to form hippuric acid, or increased hepatic N-acetyltransferase activity (Grantham et al., 1979). The capacity to acetylate and excrete 2,4-toluenediamine is less in rats than mice, and is minimal in humans and dogs (Glinsukon et al., 1975). The primary route of elimination in mice, including biliary excretion, was gastrointestinal tract within a few hours of oral and intravenous administration of ^{14}C -2,4-toluenediamine (Unger et al., 1980).

2,4-Toluenediamine was carcinogenic following dietary administration to mice and rats at levels ≥ 79 ppm, producing a significant increase in the incidence of a large variety of tumor types, including liver (NCI, 1979; Ito et al., 1969; Weisburger et al., 1978; Du Pont, 1974), mammary gland (NCI, 1979; Du Pont, 1974), subcutaneous fibromas (NCI, 1979; Weisburger et al., 1978), lung (Du Pont, 1974), lymphomas (NCI, 1979; Weisburger et al., 1978) and leukemia (NCI, 1979). Liver tumors are the most prevalent tumor type and have been observed in both rats and mice. The results of mutagenicity testing were strongly positive in bacterial assays after metabolic activation, but were inconclusive in mammalian in vitro assays. 2,4-Toluenediamine did not cause dominant lethal mutations in mice (Soares and Lock, 1980) or rats (Burnett et al., 1977) in single dose studies, but does depress DNA synthesis in mouse testes (Greene et al., 1981). There was increased mortality and a significant decrease in the number of viable litters in mice treated orally with 2,4-toluenediamine at 150 mg/kg during pregnancy (Smith, 1983). Although not statistically significant, there was a decreased sperm count in workers occupationally exposed to 2,4-toluenediamine and an increase in the spontaneous abortion rates of the wives of exposed workers (Olin Corp., 1980). In a larger follow-up study, however, no adverse reproductive effects of exposure were reported (Hamill et al.,

1982). After chronic dietary exposure to ≥ 79 ppm, 2,4-toluenediamine was hepatotoxic to rats (Du Pont, 1974; Ito et al., 1969; Weisburger et al., 1978; NCI, 1979) and mice (NCI, 1979). 2,4-Toluenediamine was nephrotoxic to rats at dietary levels ≥ 79 ppm (Du Pont, 1974; NCI, 1979). Increased levels of BUN and increased serum levels of liver enzymes were observed in occupationally exposed men, but the differences from control levels were not statistically significant (Olin Corp., 1980). Dietary administration of 2,4-toluenediamine to rats and mice resulted in striking dose-related decreases in growth rates beginning early in the treatment schedule (NCI, 1979; Ito et al., 1969; DuPont, 1974). Because no food consumption data were reported in these studies, it is not possible to determine if the poor growth rates were due to the toxicity of the chemical or to food refusal.

A q_1^* of $3.2 \text{ (mg/kg/day)}^{-1}$ was derived based on the incidence of mammary tumors and subcutaneous fibromas in female rats in the NCI (1979) study. An RQ of 100 was based on the increased mortality of rats in the NCI (1979) study. A $1/ED_{10} \text{ (F)}$ of $6.48 \text{ (mg/kg/day)}^{-1}$ was derived based on the incidence of mammary tumors in female rats. 2,4-Toluenediamine, which has an EPA carcinogenicity classification of B2 (U.S. EPA, 1984), is placed in Potency Group 3 and has a medium ranking under CERCLA.

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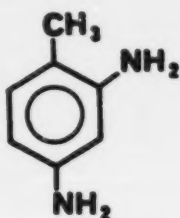
LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
BCF	Bioconcentration factor
BUN	Blood urea nitrogen
CAS	Chemical Abstract Service
CS	Composite score
DNA	Deoxyribonucleic acid
K _{OC}	Octanol/water partition coefficient
K _{OW}	Soil sorption coefficient
LD ₅₀	Median lethal dose
MED	Minimum effective dose
ND	Nondetectable
ppm	Parts per million
RQ	Reportable quantity
RV _d	Dose-rating value
RV _e	Effect-rating value
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
TWA	Time-weighted average

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Synonyms for 2,4-toluenediamine include 4-methyl-1,3-benzenediamine (9CI), toluene-2,4-diamine, meta-tolylenediamine, MTD, meta-toluylenediamine and others. The structure, molecular weight, empirical formula and CAS Registry number of 2,4-toluenediamine are listed below:



Molecular weight: 122.2

Empirical formula: $C_7H_{10}N_2$

CAS Registry number: 95-80-7

1.2. PHYSICAL AND CHEMICAL PROPERTIES

2,4-Toluenediamine is a colorless, crystalline solid (Hawley, 1981).

Selected physical properties are listed below:

Melting point:	99°C	Hawley, 1981
Boiling point:	280°C	Hawley, 1981
Vapor pressure mm Hg:		
at 20°C	5.52×10^{-5}	Lyman et al., 1982
at 106.5°C	1	Perry and Green, 1984; OHM/TADS, 1985
at 137.2°C	5	Perry and Green, 1984
Water solubility:	7.47 g/l	Lyman et al., 1982
Log K_{ow} :	0.337	U.S. EPA, 1985a
Nonaqueous solubility:	soluble in alcohol and ether; hot benzene	Hawley, 1981 Weast, 1973
Henry's Law Constant:	1.2×10^{-6} atm-m ³ /mol	Lyman et al., 1982

2,4-Toluenediamine undergoes chemical reactions typical of amines, including salt formation, alkylation, amide formation (Morrison and Boyd, 1973), acylation, isocyanide formation, di- and tetra-azotization, oxidation by neutral and basic permanganate and hydrolysis in the presence of ammonium bisulfate yielding methylresorcinol (OHM/TADS, 1985). Ring substitution reactions occur with the amine groups directing ortho- and para- (Morrison and Boyd, 1973) with substitution occurring predominantly at the 3 and 5 positions.

1.3. PRODUCTION DATA

The American companies that produced or imported 2,4-toluenediamine in 1977, their locations and production or importation volumes are listed in Table 1-1 (U.S. EPA, 1977). Air Products and Chemicals, Inc. of Pasadena, TX (ACS, 1985-1986; SRI, 1985); ICI Americas, Inc. of Geismar, LA; Mobay Chemical Corp. of Cedar Bayou, TX and New Martinsville, WV; and Olin Corp. of Lake Charles, LA (SRI, 1985) are listed as producers of 2,4-toluenediamine. Olin Corp., Rubicon Chemicals, Inc. and Union Carbide Corp. are listed as 1984 producers of 2,4-toluenediamine (USITC, 1985).

Production volumes of 2,4-toluenediamine in recent years have been reported as follows (USITC, 1981, 1982, 1983, 1984b, 1985):

<u>Year</u>	<u>Production (millions of pounds)</u>
1984	186.7
1983	202.2
1982	162.1
1981	205.0
1980	243.7

In 1983, U.S. imports of 2,4-toluenediamine were 226,060 pounds (USITC, 1984a).

TABLE 1-1

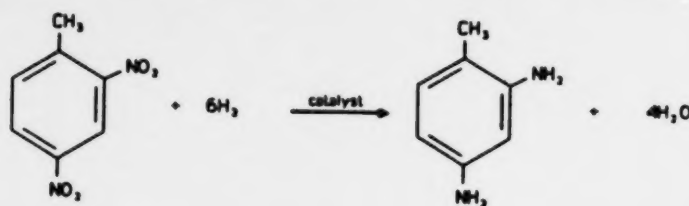
Production/Importation Data from the TSCA Production File*

Manufacturer	Location	Production/ Importation Volume (millions of pounds)
Ugine Kuhlmann of America, Inc.	Bergen, NJ	NR (I)
BASF Wyandotte Corp.	Geismar, LA	10-50 (P)
Allied Chemical Corp.	Moundsville, WV	NR (P)
Olin Corp.	Brandenburg, KY	10-50 (P)
Olin Corp.	Lake Charles, LA	50-100 (P)
Mobay Chemical Corp.	Pittsburgh, PA	NR (I)
American Cyanamid Corp.	Bound Brook, NJ	0.001-0.01 (P)
Air Products & Chemicals, Inc.	Pasadena, TX	50-100 (P)
GAF Corp.	New York, NY	0.001-0.01 (I)
E.I. DuPont de Nemours & Co.	Deepwater, NJ	10-50 (P)
NR (confidential)	NR (confidential)	0.01-0.1 (NS)

*Source: U.S. EPA, 1977

I = Importer; P = producer; NS = not specified as to importer or producer;
 NR = not reported

2,4-Toluenediamine is produced by the catalytic hydrogenation of 2,4-dinitrotoluene using a variety of temperatures, pressures and solvents, and supported noble metal catalysts, or Raney or supported nickel catalysts. Continuous, stirred flow, liquid-phase reactors are used in most commercial processes (Milligan and Gilbert, 1978). The reaction is described below:



According to Hawley (1981), m-dinitrotoluene is reduced to 2,4-toluenediamine by iron and hydrochloric acid. 2,4-Toluenediamine is usually produced as a mixture of 2,4- (80%) and 2,6-toluenediamine (20%). Smaller quantities are produced as a 65/35 mixture of 2,4- and 2,6-toluenediamine (OHM/TADS, 1985).

1.4. USE DATA

2,4-Toluenediamine is used primarily in the production of toluene diisocyanate (Milligan and Gilbert, 1978; Hawley, 1981). Intermediates in dye (Hawley, 1981; Milligan and Gilbert, 1978) and heterocyclic compound (Milligan and Gilbert, 1978) production are synthesized from 2,4-toluenediamine. Miscellaneous uses of 2,4-toluenediamine include enhancement of thermal stability in polyamides, fatigue resistance and dyeability in fibers, and the preparation of impact resistant resins, polyimides with superior wire coating properties, benzimidazolethiols (antioxidants), hydraulic fluids, urethane foams, fungicide stabilizers and sensitizers for explosives (Milligan and Gilbert, 1978). It is also used to prepare direct oxidation black, a dye for hair and furs (Hawley, 1981), and to prepare dyes for leather (OHM/TADS, 1985).

1.5. SUMMARY

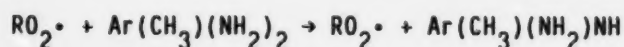
2,4-Toluenediamine undergoes reactions typical of amines, including salt formation, alkylation, amide formation (Morrison and Boyd, 1973), acylation, isocyanide formation, di- and tetra-azotization, oxidation by neutral and basic permanganate and hydrolysis in the presence of ammonium bisulfate yielding methylresorcinol (OHM/TADS, 1985). In 1984, 186.7 million pounds of 2,4-toluenediamine were produced (USITC, 1984b). 2,4-Toluenediamine is produced by the catalytic hydrogenation of 2,4-dinitrotoluene using a variety of temperatures, pressures and solvents, and supported noble metal catalysts, or Raney or supported nickel catalysts. Continuous, stirred flow, liquid-phase reactors are used in most commercial processes (Milligan and Gilbert, 1978). 2,4-Toluenediamine is usually produced as a mixture of 2,4- (80%) and 2,6-toluenediamine (OHM/TADS, 1985), and is used primarily in the production of toluene diisocyanate (Milligan and Gilbert, 1978; Hawley, 1981). It is also used in the production of dyes (Hawley, 1981) and in the preparation of impact-resistant resins, polyimides with superior wire-coating properties, benzimidazolethiols (antioxidants), hydraulic fluids, urethane foams, fungicide stabilizers and sensitizers for explosives (Milligan and Gilbert, 1978).

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. WATER

2.1.1. Hydrolysis. Pertinent hydrolysis data for 2,4-toluenediamine could not be located in the available literature as cited in the Appendix. Lyman et al. (1982), however, classified aromatic amines as generally resistant to hydrolysis under environmental conditions.

2.1.2. Oxidation. Singlet oxygen ($^1O_2$), alkyl peroxy radicals ($RO_2^\bullet$), alkoxy radicals ($RO^\bullet$) and hydroxyl radicals ($\bullet OH$), which are generated in water by sunlight, are capable of oxidizing 2,4-toluenediamine (Mabey et al., 1981). The oxidation reaction initiated by $RO_2^\bullet$ is shown below.



The second amine group is presumably also subject to oxidation in this manner. The reaction for aniline ($ArNH_2$) is rapid, with a half-life of <2 days (Mabey et al., 1981). In sunlit waters, therefore, oxidation of 2,4-toluenediamine by $RO_2^\bullet$ may be a significant fate process.

2.1.3. Photolysis. 2,4-Toluenediamine has an absorption maximum at 294 nm ($\epsilon=2891$ l/mol-cm) in methanol (Sadler, 1960). It is possible, therefore, that 2,4-toluenediamine photolyzes in water under environmental conditions. In the absence of experimental data, however, no statements can be made concerning the rate of photolysis.

2.1.4. Microbial Degradation. Matsui et al. (1975) observed a 45% decline in the theoretical organic carbon of 2,4-toluenediamine after 4 hours contact with activated sludge in a fill and draw apparatus. The experimental method does not prevent volatilization or hydrolysis, but since

2,4-toluenediamine is not expected to undergo extensive volatilization or hydrolysis, biodegradation is most likely responsible for this decline. The presence of amine groups confers some degree of biodegradability on aromatic rings (Hutzinger and Veerkamp, 1981), and Lyman et al. (1982) classified aniline as a relatively biodegradable compound. Sasaki (1978) listed 2-methylaniline and 4-methylaniline as substances confirmed to be biodegraded in screening tests.

2.1.5. Transport.

2.1.5.1. VOLATILIZATION -- Based on an estimated water solubility of 7.47 g/l and an estimated vapor pressure of 7.2×10^{-8} atm at 20°C, a Henry's Law constant of 1.2×10^{-9} atm·m³/mol was estimated for 2,4-toluenediamine (Lyman et al., 1982). This value is ~10 orders of magnitude less than that for water under similar conditions (Lyman et al., 1982). Volatilization, therefore, is expected to be insignificant in the environment.

2.1.5.2. ADSORPTION -- A $\log K_{oc}$ of 1.56 was estimated from an estimated $\log K_{ow}$ of 0.337 (U.S. EPA, 1985a). According to Kenaga (1980), a K_{oc} value of this magnitude indicates that 2,4-toluenediamine should be moderately to highly mobile and should, therefore, leach to groundwater.

2.1.5.3. BIOCONCENTRATION -- An experimental BCF of 91 was determined for an unspecified isomer of toluediamine using fathead minnows exposed for 32 days (Veith et al., 1979). Using the estimated $\log K_{ow}$ of 0.337 for 2,4-toluenediamine, a BCF of 1.06 was estimated (Lyman et al., 1982). Based on these BCF values, 2,4-toluenediamine is not expected to bioconcentrate significantly in aquatic organisms.

2.2. AIR

Pertinent data regarding the atmospheric chemistry of 2,4-toluenediamine could not be located in the available literature as cited in the Appendix. An atmospheric half-life of 7.99 hours has been estimated for the reaction of 2,4-toluenediamine with hydroxyl radicals (U.S. EPA, 1985b). 2,4-Toluenediamine has an absorption maximum at 294 nm ($\epsilon=2891$ l/mol-cm) in methanol (Sadler, 1960). It is possible, therefore, that 2,4-toluenediamine photolyzes in the atmosphere. In the absence of experimental data, however, no statements concerning the rate of photolysis can be made.

2.3. SOIL

2.3.1. Microbial Degradation. Pertinent data regarding the biodegradation of 2,4-toluenediamine by soil dwelling microorganisms could not be located in the available literature as cited in the Appendix. Matsui et al. (1975) observed a 45% decline in the theoretical organic carbon of 2,4-toluenediamine after 4 hours contact with activated sludge in a fill and draw apparatus. The experimental method does not prevent volatilization or hydrolysis, but since 2,4-toluenediamine is not expected to undergo extensive volatilization or hydrolysis, biodegradation is most likely responsible for this decline. The presence of amine groups confers some degree of biodegradability on aromatic rings (Hutzinger and Veerkamp, 1981), and Lyman et al. (1982) classified aniline as a relatively biodegradable compound. Sasaki (1978) listed 2-methylaniline and 4-methylaniline as confirmed biodegradable substances. 2,4-Toluenediamine is therefore expected to biodegrade in soil. It should be noted, however, that because of the specificity of enzymatic reactions, predictions of biodegradability based on comparisons

with similar compounds are tenuous. Also, conclusions concerning biodegradation by soil microbes based on the behavior of sludge microbes should be considered tenuous because of differences between microorganisms in activated sludge and soil.

2.3.2. Photolysis. 2,4-Toluenediamine has an absorption maximum at 294 nm ($\epsilon=2891$ l/mol-cm) in methanol (Sadler, 1960). It is possible, therefore, that 2,4-toluenediamine photolyzes in soil under environmental conditions. In the absence of experimental data, however, no statements can be made concerning the rate of photolysis.

2.3.3. Adsorption-Leaching. A $\log K_{oc}$ of 1.56 was estimated for 2,4-toluenediamine from an estimated $\log K_{ow}$ of 0.337 (U.S. EPA, 1985a). According to Kenaga (1980), a $\log K_{oc}$ value of this magnitude indicates that 2,4-toluenediamine should be moderately to highly mobile in soils, and should leach to groundwater. This conclusion, however, is based solely on physical interactions. Aromatic amines are known to form covalent bonds to humic materials (quinone-like compounds) followed by slow oxidation (Parris, 1980). Although no data were available indicating that it does so, 2,4-toluenediamine may also react in this way and will most likely remain in the soil much longer than the purely physical interactions suggest.

2.3.4. Volatilization. Pertinent data regarding the volatilization of 2,4-toluenediamine from soil could not be located in the available literature as cited in the Appendix. Based on the high estimated water solubility and very low estimated vapor pressure of 2,4-toluenediamine (Lyman et al., 1982), however, little or no volatilization is expected.

2.4. SUMMARY

According to Lyman et al. (1982), 2,4-toluenediamine is not expected to hydrolyze significantly. Based on an estimated Henry's Law constant of 1.2×10^{-9} atm·m³/mol (Lyman et al., 1982), 2,4-toluenediamine will not volatilize from water. The very low estimated vapor pressure of 2,4-toluenediamine at 20°C and its high estimated water solubility suggest that little or no volatilization from soil will occur. Aqueous photooxidation (Mabey et al., 1981) and aerobic biodegradation (Matsui et al., 1975) are expected to be the major factors in the environmental fate of 2,4-toluenediamine. Atmospheric and aqueous photolysis may occur, given the absorption maximum of 294 nm ($\epsilon=2891$ l/mol·cm) (Sadler, 1960). 2,4-Toluenediamine has an estimated log K_{oc} value of 1.56 (Lyman et al., 1982); it is not expected to bind tightly to soil (Kenaga, 1980) and should leach to groundwater. This conclusion, however, is based solely on physical interactions. Aromatic amines are known to form covalent bonds to humic materials (quinone-like compounds) followed by slow oxidation (Parris, 1980). Although no data were available indicating that it does so, 2,4-toluenediamine may also react in this way and will most likely remain in the soil much longer than the purely physical interactions suggest. According to Veith et al. (1979), an unspecified isomer of toluediamine exhibits a BCF of 91, and the estimated BCF for 2,4-toluenediamine is 1.06 (Lyman et al., 1982). These BCF values indicate that bioconcentration will not be significant.

3. EXPOSURE

3.1. WATER

Pertinent monitoring data for 2,4-toluenediamine in water could not be located in the available literature as cited in the Appendix. 2,4-Toluenediamine was identified but not quantified in wastewater samples from an unspecified industry (Zygmunt et al., 1983). The amount of 2,4-toluenediamine released to water as a result of the production process was estimated to be 13.6 million pounds (Johnson et al., 1980). Monitoring data are required before an average daily intake value can be calculated.

3.2. FOOD

Since pertinent monitoring data for 2,4-toluenediamine in food could not be located in the available literature, no estimate of the average daily intake can be made. Boil-in-bags and retortable pouches were filled with Milli-Q water and boiled at 212°F for 2 hours, and the water was analyzed for 2,4-toluenediamine. Apparent migration of 2,4-toluenediamine from the bags and pouches into the water averaged 0.25 ppb and 2.875 ppb, respectively (Snyder and Breder, 1982). It is possible that using these containers to cook food may result in residues of 2,4-toluenediamine in food, but such residues are expected to be low.

3.3. AIR

Pertinent data regarding the monitoring of 2,4-toluenediamine in ambient air could not be located in the available literature as cited in the Appendix. The amount of 2,4-toluenediamine released to the air as a result of its production in 1977 was estimated to be 1.54 million pounds (Johnson et al., 1980). Monitoring data are required before an average daily intake can be calculated, and industrial monitoring data were available. The concentration of 2,4-toluenediamine in samples around vertical or horizontal

coating machines during the production of polyurethane-coated wire ranged from ND(<0.0005)-8.13 mg/m³, with an average concentration of 0.99 mg/m³ (Rosenberg, 1984). The highest concentration observed was detected in the exhaust ventilation. (Three unspecified varnishes were used in the coating of the wires.) 2,4-Toluenediamine concentrations in samples of the working atmosphere of the Olin Chemical Company, a producer of 2,4-toluenediamine, ranged from 0.008-0.39 mg/m³ in one survey and from ND-0.038 mg/m³ in a second survey (Ahrenholz, 1980).

3.4. SUMMARY

The amount of 2,4-toluenediamine released to water as a result of its production in 1977 was estimated to be 13.6 million pounds (Johnson et al., 1980). 2,4-Toluenediamine was found to leach from boil-in-bags and retortable pouches into water, achieving average concentrations of 0.25 ppb and 2.875 ppb in the water, respectively (Snyder and Breder, 1982). The amount of 2,4-toluenediamine released to the air as a result of its production was estimated to be 1.54 million pounds (Johnson et al., 1980). Additional monitoring data are required before average daily intake values of 2,4-toluenediamine for the general population from exposure to water, air and food can be estimated. The concentration of 2,4-toluenediamine in air samples taken during the production of polyurethane-coated wire ranged from ND (0.0005) to 8.13 mg/m³ with an average concentration of 0.99 mg/m³ (Rosenberg, 1984), and with the highest concentration occurring in the exhaust ventilation. 2,4-Toluenediamine concentrations in samples of the working atmosphere of the Olin Chemical Company, a producer of 2,4-toluenediamine, ranged from 0.008-0.39 mg/m³ in one survey and from ND to 0.038 mg/m³ in a second survey (Ahrenholz, 1980).

4. PHARMACOKINETICS

4.1. ABSORPTION

Based on the amount of radioactivity recovered from the urine and feces of male Sprague-Dawley rats (three/group) within 73 hours of gavage treatment with 0.5 or 50 mg/kg of ^{14}C -toluene-2,4-diamine (uniformly ring labeled), Sauerhoff et al. (1977) concluded that ~90% of the ingested 2,4-toluenediamine was absorbed. Oral administration of an aqueous solution of 300 mg/kg of 2,4-toluenediamine to 26 male rats (strain unspecified) killed in pairs at 0, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24 hours after dosing resulted in increased blood levels of 2,4-toluenediamine within 1 hour of dosing and peak blood levels of 2,4-toluenediamine within 2 hours of dosing (Du Pont, 1974).

Pertinent data regarding the absorption of 2,4-toluenediamine by inhalation could not be located in the available literature as cited in the Appendix.

Marzulli et al. (1981) reported that, of topically applied ^{14}C -2,4-toluenediamine (uniformly ring labeled), $53.8 \pm 15.4\%$ (in male and female rhesus monkeys) and $23.7 \pm 16.1\%$ (in male humans) penetrated the skin within 24 hours. As reviewed by Dow Chemical Co. (1983), 16, 24 and 28% of the radioactivity from ^{14}C -2,4-toluenediamine applied to the abdominal skin of three rhesus monkeys (sex unspecified) was recovered from the urine within 5 days of dosing (Marzulli and Greene, 1978).

4.2. DISTRIBUTION

After a single oral administration of 300 mg/kg of 2,4-toluenediamine to male rats (strain unspecified), the highest concentrations of 2,4-toluenediamine and an unspecified metabolite during the 24 hours after dosing occurred in the blood, liver and kidneys and the lowest concentrations

occurred in the muscle, heart and brain (Du Pont, 1974). No other tissues were analyzed. Although 2,4-toluenediamine did not accumulate in any tissue or organ, the metabolite was still detectable in the tissue and blood of dosed rats 24 hours after administration.

Groups of nine male Fischer 344 rats were given an intraperitoneal injection of 1, 10 or 100 mg/kg of ^{14}C -2,4-toluenediamine (methyl labeled) (Allied Corp., 1982). Groups of three rats were killed 0.5, 1.5 and 4.5 hours after dosing. Binding of radioactivity to DNA and protein occurred in the testes and muscle tissue only at the highest dose tested. Binding of radioactivity to DNA, RNA and protein in the liver, however, occurred in a time- and dose-related fashion.

After intraperitoneal administration of 77 mg/kg of ^{14}C -2,4-toluenediamine (uniformly ring labeled) to groups of three or four male Fischer rats (235-255 g) and male NIH Swiss mice (27-32 g), the highest concentration of radioactivity occurred in the liver and kidneys of both rats and mice (Grantham et al., 1979) (Tables 4-1 and 4-2). Of the organs evaluated (liver, kidney, lung, spleen, heart and testis), the testes had the lowest concentration of radioactivity.

Male B6C3F1 mice (30-35 g) were injected intraperitoneally with 0.667 mg/kg of ^{14}C -2,4-toluenediamine (methyl labeled) and were chloroformed in groups of five at 0.5, 1, 2, 6, 16 and 24 hours after treatment (Unger et al., 1980). As indicated in Table 4-3, the highest concentration of radioactivity occurred in the liver, kidneys, adrenals, gastrointestinal tract and associated lymph nodes, and a relatively high concentration of radioactivity occurred in the eyes and lungs. In most organs (liver, adrenals) the peak concentration of radioactivity occurred 1 hour after dosing, but in the kidneys, testes, lungs, muscle and blood, the peak concentration

TABLE 4-1

Distribution of Radioactivity in Fischer Rats 1 and 24 Hours After
Intraperitoneal Injection of 77 mg/kg ^{14}C -2,4-Toluenediamine^a

Tissue	Radioactivity ($\mu\text{g/g}$ wet weight + S.E.)	
	1 Hour ^b (n=4)	24 Hours (n=4)
Liver	52.4 $\pm$ 1.1	34.1 $\pm$ 2.1
Kidney	79.1 $\pm$ 4.5	44.8 $\pm$ 5.6
Lung	14.9 $\pm$ 0.7	13.3 $\pm$ 1.7
Spleen	13.2 $\pm$ 0.5	13.5 $\pm$ 0.3
Heart	11.7 $\pm$ 0.6	7.9 $\pm$ 0.9
Testis	10.2 $\pm$ 0.1	3.4 $\pm$ 0.3
Blood ^c	13.2 $\pm$ 0.6	13.1 $\pm$ 1.5
Plasma ^c	17.5 $\pm$ 0.8	11.2 $\pm$ 1.9

^aSource: Grantham et al., 1979

^bThe values in this column were obtained 1 hour after an unspecified amount of ^{14}C -2,4-toluenediamine was administered intravenously.

^c $\mu\text{g/ml}$

S.E. = Standard error

TABLE 4-2

Distribution of Radioactivity in NIH Swiss Mice 24 and 48 Hours After
Intraperitoneal Injection of 77 mg/kg ^{14}C -2,4-Toluenediamine^a

Tissue	Radioactivity ($\mu\text{g/g}$ wet weight + S.E.)	
	24 Hours (n=4)	48 Hours (n=4)
Liver	11.8 $\pm$ 1.3	7.9 $\pm$ 0.6
Kidney	6.8 $\pm$ 1.3	4.2 $\pm$ 0.5
Lung	4.7 $\pm$ 0.3	3.1 $\pm$ 0.2
Spleen	5.7 $\pm$ 1.4	6.0 $\pm$ 1.2
Heart	3.1 $\pm$ 0.4	2.7 $\pm$ 0.2
Testis	2.1 $\pm$ 0.2	1.2 $\pm$ 0.1
Blood ^b	1.8 $\pm$ 0.2	1.8 $\pm$ 0.4
Plasma ^b	1.6 $\pm$ 0.3	1.1 $\pm$ 0.3

^aSource: Grantham et al., 1979

^b $\mu\text{g/ml}$

S.E. = Standard error

TABLE 4-3

Distribution of Radioactivity in Tissues of Mice at Indicated Intervals After Intraperitoneal Administration of 0.667 mg/kg 2,4-[¹⁴C]-Toluenediamine^a

Organ	Radioactivity In nanogram-equivalents of 2,4-toluenediamine/g of tissue											
	0.5 Hour		1.0 Hour		2.0 Hours		6.0 Hours		16.0 Hours		24.0 Hours	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Liver	999	47	1484	162	712	52	236	12	94	4	83	5
Spleen	82	9	126	31	76	11	46	15	23	2	24	2
Stomach ^b	658	106	997	291	523	112	416	205	32	6	53	13
Small Intestine ^b	1567	312	2751	1093	2168	610	674	212	37	2	52	9
Large Intestine ^b	150	14	1356	489	2762	772	2466	753	84	16	311	55
Pancreas	110	7	155	31	97	18	54	10	20	2	16	2
Kidneys	701	65	623	80	222	32	138	24	46	2	36	3
Adrenals	258	46	1286	488	399	175	NR	NR	NR	NR	NR	NR
Gonads	78	25	56	9	37	6	14	4	5	NR	5	1
Epididymis	132	27	78	25	68	7	26	6	8	2	13	4
Thyroid	NR	NR	233	25	224	7	49	6	NR	NR	NR	NR
Thymus	87	7	96	26	69	7	36	10	15	1	12	3
Salivary gland	142	9	177	34	134	16	48	3	31	1	22	3
Lymph nodes ^c	433	132	614	313	318	114	400	237	NR	NR	NR	NR
Heart	82	8	83	12	58	9	20	4	14	1	11	1
Aorta	116	13	169	31	210	39	NR	NR	NR	NR	NR	NR
Lungs	300	29	294	80	169	24	63	9	43	3	31	2
Marrow ^d	82	9	113	33	74	21	19	3	10	NR	9	1
Muscle	76	18	60	14	36	5	11	1	61	NR	7	1

TABLE 4-3 (cont.)

Organ	Radioactivity in nanogram-equivalents of 2,4-toluenediamine/g of tissue											
	0.5 Hour		1.0 Hour		2.0 Hours		6.0 Hours		16.0 Hours		24.0 Hours	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Brain	39	2	47	7	29	3	6	1	4	NR	3	NR
Eyes	254	17	469	132	382	55	176	18	250	22	153	9
Skin	105	6	162	65	48	3	39	2	38	13	25	4
Fat ^c	69	15	75	21	44	10	40	8	7	1	7	2
Blood	123	12	100	13	73	21	21	4	11	NR	8	1

^aSource: Unger et al., 1980^bIncludes contents^cLymph nodes were excised from the serosal surface of the small intestine.^dBone marrow was taken from the sternum.^eFat was taken from the suprasternal fat pad.

NR = Not reported: Level of radioactivity too low to be measured accurately

occurred 0.5 hours after dosing. Elimination of radioactivity from the tissues was biphasic (Section 4.4.). During the α phase of elimination, the half-life of radioactivity in the liver, kidney and blood was 0.89, 0.43 and 1.51 hours, respectively; during the β phase of elimination, 11.7, 9.1 and 12.6 hours, respectively (Table 4-4).

4.3. METABOLISM

The in vivo metabolism of 2,4-toluenediamine has been studied in several species. Waring and Pheasant (1976) treated female Wistar rats, female New Zealand rabbits and Dunkin-Harvey guinea pigs by gavage with 50 mg/kg. Urine was collected for 48 hours and analyzed for metabolites. Nine metabolites were isolated and characterized (Table 4-5). Metabolites I-VI listed in Table 4-5 were ring-hydroxylated and N-acetylated derivatives, while metabolites VII-IX were glucuronide conjugates of unknown structure. Quantitative differences were observed among the three species. In the proposed metabolic pathway (Figure 4-1), 2,4-toluenediamine is hydroxylated at the 3, 5 or 6 position to metabolites I, II or III, respectively. N-acetylation of the 4-amino group of metabolites I and II yields metabolites IV and VI, respectively. No N-acetylated derivative of metabolite III was found.

Grantham et al. (1979) found different metabolites in the urine collected for 24 hours following intraperitoneal dosing of male Fischer rats and male Swiss mice with 77 mg/kg of 2,4-toluenediamine. Free and water soluble metabolites accounted for ~50% of the dose given to either species, while glucuronide and sulfate conjugates accounted for ~18% of the dose for rats and ~27% of the dose for mice. Nine metabolites were isolated from the free fraction and were identified as indicated in Table 4-6. In contrast to the results of Waring and Pheasant (1976), no ring-hydroxylated metabolites were formed.

TABLE 4-4

Reported Half-Lives for Various Organs From Mice Treated
Intraperitoneally with ^{14}C -2,4-Toluenediamine*

Organ	Half-Lives (hours)	
	α Phase	β Phase
Liver	0.89	11.7
Kidney	0.43	9.1
Blood	1.51	12.6
Muscle	NR	23.9
Skin	NR	29.2

*Source: Unger et al., 1980

NR = Not reported

TABLE 4-5

Metabolites of 2,4-Toluenediamine in the Urine of Female
New Zealand Rabbits, Wistar Rats and Dunkin-Harvey
Guinea Pigs Treated Orally with 50 mg/kg^a

Metabolite	Percentage Dose ^b		
	Rabbits	Rats	Guinea Pigs
2,4-Toluenediamine	trace	1.3	trace
I. 3-hydroxy-2,4-toluene-diamine	10	8	trace
II. 5-hydroxy-2,4-toluene-diamine	22	12	9
III. 6-hydroxy-2,4-toluene-diamine	trace	5	trace
IV. 3-hydroxy-4-acetamido-2-aminotoluene	10	18	trace
V. aromatic amines and phenols (not further characterized)	0	trace	trace
VI. 5-hydroxy-4-acetamido-2-aminotoluene	6	14	17
VII	10	16	15
VIII	32	12	46
IX	2	6	4

^aSource: Waring and Pheasant, 1976

^bThe results are an average of 10 experiments.

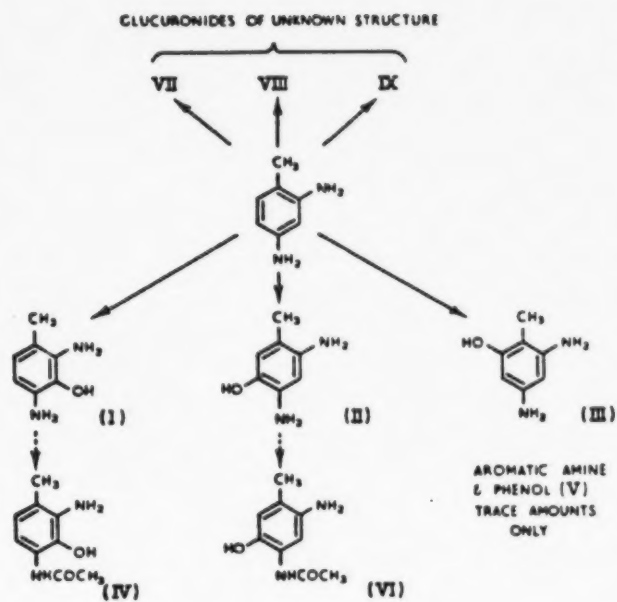


FIGURE 4-1
Metabolic Pathway of 2,4-Toluenediamine
Source: Waring and Pheasant, 1976

TABLE 4-6

Metabolites of 2,4-Toluenediamine in the Urine of Male Fischer
Rats and Swiss Mice Treated Intraperitoneally with 77 mg/kg*

Metabolite	Percentage of Dose	
	Rats	Mice
2,4-Diacetylaminobenzoic acid methyl ester	0.6	1.1
2,4-Diaminobenzoic acid methyl ester	2.7	5.4
2,4-Toluenediamine	0.4	0.4
4-Acetamido-2-aminotoluene	5.7	2.1
2-Acetamido-4-aminotoluene	0.8	0.5
2,4-Diacetamidotoluene	2.6	0.3
α -Hydroxy-2,4-diacetamidotoluene (tentative)	1.2	1.2
α -Hydroxy-2,4-toluenediamine	0.7	0.8
Unidentified metabolite	0.3	0.4

*Source: Grantham et al., 1979

The percentages of the 4-acetamido derivative were substantially greater than the 2-acetamido derivative in both rats and mice (Grantham et al., 1979). Similarly, Waring and Pheasant (1976) found relatively high percentages of hydroxylated-4-acetamido-2-aminotoluene derivatives in the urine of rats, rabbits and guinea pigs; no hydroxylated 2-acetamido-4-aminotoluene derivatives were found. These observations are consistent with in vitro results of Glinsukon et al. (1975), who incubated 2,4-toluenediamine with liver cytosolic fractions from several species in the presence of acetyl-CoA. 4-Acetamido-2-aminotoluene was the major in vitro metabolite, while no 2-acetamido-4-aminotoluene was found. Small amounts of 2,4-diacetamidotoluene were formed and may have arisen from small amounts of 2-acetamido-4-aminotoluene. The results suggested that N-acetylation occurs preferentially at the 4-amino group of 2,4-toluenediamine. Species differences in the ability of liver cytosol to N-acetylate 2,4-toluenediamine are indicated in Table 4-7. Compared with the liver cytosol fraction from rats, guinea pigs, rabbits, mice and hamsters, human and dog liver cytosol had insignificant or no acetylating activity. These in vitro data suggest that different metabolic pathways operate in humans and dogs than in rodents.

4.4. EXCRETION

Groups of two male and two female Sprague-Dawley rats were injected intravenously with 0.5 or 50.0 mg/kg of ^{14}C -2,4-toluenediamine and the urine and feces were collected at 12- or 24-hour intervals for 72 hours after dosing (Sauerhoff et al., 1977). Of the administered radioactivity, >70% was recovered from the urine within 72 hours; <1.5% of the administered radioactivity remained in the rats 72 hours after dosing. As indicated in Table 4-8, the relative amounts of radioactivity recovered from the urine and feces are dependent upon the dose level of ^{14}C -2,4-toluenediamine

TABLE 4-7
Enzymic N-Acetylation of 2,4-Toluenediamine by Liver Cytosol
from Various Species^a

Species	Sex	Number of Animals	Enzyme Activity ^b (nmol acetamidotoluene/50 mg wet wt liver/5 min)	
			4-Acetamido-2- aminotoluene	2,4-Diacetamido- toluene
Hamster	male	5	969 $\pm$ 28	12.1 $\pm$ 2.1
Guinea pig	male	4	792 $\pm$ 66	6.4 $\pm$ 0.6
Rabbit	male	3	600 $\pm$ 35	6.2 $\pm$ 1.0
Mouse	male	5	604 $\pm$ 45	5.9 $\pm$ 1.8
Mouse	female	4	677 $\pm$ 20	7.4 $\pm$ 1.2
Rat	male	5	233 $\pm$ 6	ND
Rat	female	5	182 $\pm$ 16	ND
Man	male	3	3.0 $\pm$ 0.9	ND
Dog	male	1	0	0

^aSource: Glinsukon et al., 1975

^bEnzyme activity with standard deviation

ND = Not detectable

TABLE 4-8

Percentage of Dose in Urine and Feces of Rats Injected Intravenously
with 0.5 or 50 mg/kg ^{14}C -2,4-Toluenediamine*

Time (hours)	0.5 mg/kg		50 mg/kg	
	Urine	Feces	Urine	Feces
12	64.9 $\pm$ 4.0	NR	68.0 $\pm$ 6.8	NR
24	5.99 $\pm$ 8.31	12.2 $\pm$ 8.0	10.1 $\pm$ 7.5	12.1 $\pm$ 1.9
36	0.80 $\pm$ 0.42	NR	0.58 $\pm$ 0.10	NR
48	0.35 $\pm$ 0.13	7.86 $\pm$ 7.58	0.32 $\pm$ 0.13	2.22 $\pm$ 0.73
60	0.24 $\pm$ 0.09	NR	0.21 $\pm$ 0.09	NR
72	0.22 $\pm$ 0.10	0.28 $\pm$ 0.18	0.15 $\pm$ 0.04	0.37 $\pm$ 0.22
Total	72.5 $\pm$ 5.9	20.4 $\pm$ 4.0	79.4 $\pm$ 1.9	15.0 $\pm$ 1.4

*Source: Sauerhoff et al., 1977

NR = Not reported

administered. At the low dose, relatively more radioactivity is recovered in the urine; at the high dose, relatively more radioactivity is recovered from the feces. Sauerhoff et al. (1977) reported that plasma clearance of ^{14}C -2,4-toluenediamine was biphasic at both dose levels. At the low dose level, the plasma half-life was 0.53 ± 0.28 hours and 8.5 ± 2.7 hours for the α and β phase, respectively. At the high dose level, the plasma half-life was 0.88 ± 0.10 hours and 14.5 ± 5.9 hours for the α and β phase, respectively. The terminal phase urinary excretion half-life values ($t_{1/2\beta}$) were also different after low (0.5 mg/kg) and high (50 mg/kg) doses of ^{14}C -2,4-toluenediamine were administered intravenously: the $t_{1/2\beta}$ was 18.2 ± 6.8 hours at the low dose and 26.7 ± 14.9 hours at the high dose. Despite the lack of statistical significance in these differences, Sauerhoff et al. (1977) maintained that their data suggest that the half-life of 2,4-toluenediamine in rats is dose-dependent after intravenous injection. Dose-dependent pharmacokinetics was clearly suggested, however, by a disproportionate increase in plasma levels of radioactivity (200-fold greater in 50 mg/kg treated rats than in 0.5 mg/kg rats) relative to the magnitude of the dose difference (100-fold).

In a similar experiment, groups of three male Sprague-Dawley rats were treated by gavage with a single dose of 0.5 or 50 mg/kg of ^{14}C -2,4-toluenediamine and the urine and feces were collected for 72 hours after dosing (Sauerhoff et al., 1977) (Table 4-9). Although the relative amount of radioactivity excreted in the urine increased at the high dose level after intravenous administration, the relative amount of radioactivity excreted in the urine decreased at the high dose level after oral administration. Therefore, the oral data does not support the suggestion by Sauerhoff et al. (1977) that the half-life of 2,4-toluenediamine is dose-dependent.

TABLE 4-9

Percentage of Dose in Urine and Feces of Rats Administered
0.5 or 50 mg/kg ¹⁴C-2,4-Toluenediamine by Gavage*

Time (hours)	0.5 mg/kg		50 mg/kg	
	Urine	Feces	Urine	Feces
12	39.6±20.6	2.2±3.6	48.7±6.1	2.2±1.9
24	13.5±11.3	16.5±4.5	7.3±2.2	21.2±2.0
36	2.5±1.0	2.4±2.2	2.1±1.6	1.4±0.6
48	4.8±4.9	1.9±1.0	1.5±1.2	4.5±1.4
60	1.3±0.8	0.1±0.3	NR	NR
72	1.9±2.1	2.9±1.9	NR	NR
Total	63.6±1.7	25.1±2.5	59.9±3.3	31.2±4.9

*Source: Sauerhoff et al., 1977

NR = Not reported

A group of 26 male rats (strain unspecified) was treated orally with 300 mg/kg of ^{14}C -2,4-toluenediamine as indicated in Section 4.1. (DuPont, 1974). The rats were sacrificed in pairs 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24 hours after dosing, and the amounts of radioactivity in the blood, liver, kidney, muscle, heart and brain were determined. The half-life of 2,4-toluenediamine and an unidentified metabolite, which decreased at a rate approaching a first-order reaction, was reported to be 5 hours (Du Pont, 1974).

After intraperitoneal injection of 0.667 mg/kg of ^{14}C -2,4-toluenediamine in male B6C3F mice (see Section 4.2.), 52% of the administered dose had been recovered from the urine and 22% had been recovered from the feces within 24 hours of dosing (Unger et al., 1980). As indicated in Table 4-10, 49% of the administered dose had been recovered from the urine within 2 hours of dosing, but not until 16 hours after dosing was 20% of the administered dose recovered from the feces. Unger et al. (1980) concluded that excretion by the kidneys was the primary route for the first 2 hours after dosing, but was gradually replaced by excretion by the gastrointestinal tract, including biliary excretion. The data of Unger et al. (1980) suggest a biphasic clearance of 2,4-toluenediamine in the mouse and would necessitate the use of a 2-compartment model in pharmacokinetic calculations. Radioactivity in the expired air accounted for only 1.25% of the administered dose during 24 hours after ^{14}C -2,4-toluenediamine administration (Unger et al., 1980).

As discussed in Sections 4.2. and 4.3., groups of male rats and mice were injected intraperitoneally with 77 mg/kg of ^{14}C -2,4-toluenediamine (Grantham et al., 1979). In the mice, ~90% of the administered dose was excreted in the urine within 24 hours, but in the rats only 74% of the

TABLE 4-10
Percentage of Dose in Urine and Feces of Mice After
Intraperitoneal Injection of ^{14}C -2,4-Toluenediamine^a

Time ^b (hours)	Urine ^c	Feces
0.5	14.85 $\pm$ 5.92	NR
1.0	49.24 $\pm$ 19.89	NR
2.0	46.22 $\pm$ 14.05	NR
6.0	54.43 $\pm$ 9.01	2.34
16.0	52.40 $\pm$ 5.4	20.41
24.0	51.80 $\pm$ 2.15	22.36

^aSource: Unger et al., 1980

^bFive mice sacrificed at each time interval

^cIncludes bladder contents

NR = Not reported

administered dose was excreted in the urine within 24 hours. Grantham et al. (1979) reported that 2,4-toluenediamine was eliminated from mice within 2 days of dosing but was eliminated from rats within 6 days of dosing. One of the mechanisms reported by Grantham et al. (1979) as partially responsible for the increased rate of elimination in mice is the increased rate of conjugation of 2,4-toluenediamine metabolites with glucuronic acid, which presumably facilitates urinary excretion. Other mechanisms that may facilitate excretion of 2,4-toluenediamine and its metabolites in mice include increased activity of hepatic N-acetyltransferase and increased oxidation of the methyl group to form benzoic acid, which can then be conjugated to glycine to form hippuric acid (Williams, 1959; Grantham et al., 1979).

4.5. SUMMARY

Absorption of 2,4-toluenediamine by inhalation has not been evaluated. Absorption of 2,4-toluenediamine was ~90% after oral administration to rats (Sauerhoff et al., 1977) and ~54 and 24% after a 24-hour dermal application to monkeys and humans, respectively (Marzulli and Greene, 1978; Marzulli et al., 1981). After oral and intraperitoneal administration, the liver and kidneys are among the organs with the highest concentration of 2,4-toluenediamine in rats and mice, while the testes are among the organs with the lowest concentration (Unger et al., 1980; Allied Corp., 1982; Grantham et al., 1979; Du Pont, 1974). The half-life of 2,4-toluenediamine is different in different organs, being shortest in the kidney (9.1 hours) and longest in the skin (29.2 hours) (Unger et al., 1980). Metabolism of 2,4-toluenediamine may occur by hydroxylation of ring carbons, acetylation of amino groups and oxidation of the methyl group depending upon species and strain (Waring and Pheasant, 1976; Grantham et al., 1979). Glucuronide and sulfate conjugates are also formed. Although Du Pont (1974) reported that the rate

of elimination of 2,4-toluenediamine in rats approaches first-order kinetics, Sauerhoff et al. (1977) reported biphasic elimination in rats and Unger et al. (1980) reported biphasic elimination in mice. Elimination of a single oral dose (77 mg/kg) is virtually complete in mice in 2 days but not completed in rats until 6 days after dosing (Grantham et al., 1979). The increased clearance of 2,4-toluenediamine in mice as compared with rats may be due to increased conjugation of 2,4-toluenediamine metabolites to glucuronic acid, increased oxidation rates to form benzoic acid, which can be conjugated to glycine to form hippuric acid, or increased hepatic N-acetyltransferase activity (Grantham et al., 1979). The capacity to acetylate and excrete 2,4-toluenediamine, is less in rats than in mice and is minimal in humans and dogs (Glinsukon et al., 1975). Excretion by the kidneys is replaced by excretion by the gastrointestinal tract (including biliary excretion) as the main route within a few hours of oral and intravenous administration of 2,4-toluenediamine (Unger et al., 1980).

5. EFFECTS

5.1. CARCINOGENICITY

Groups of 12 male Wistar rats were exposed to 600 or 1000 ppm of dietary 2,4-toluenediamine for 33-34 weeks (Ito et al., 1969). The control group consisted of six male rats fed the same diet without added 2,4-toluenediamine. There was a striking dose-related decrease in body weight gain in the treated rats compared with controls; after 4 weeks they stopped gaining weight and after 8 weeks, they gradually lost weight until they died or were killed. Food consumption data were not reported. Histological examination was limited to the liver. After 33 weeks of treatment, 9/9 surviving rats exposed to 1000 ppm of 2,4-toluenediamine had hepatocellular carcinoma, accompanied by nodular hyperplasia, cholangiofibrosis and fatty changes. Cirrhosis was minimal. After 34 weeks of treatment, 7/11 surviving rats exposed to 600 ppm of dietary 2,4-toluenediamine had hepatocellular carcinoma, accompanied by nodular hyperplasia, minimal fatty changes and cholangiofibrosis. No cirrhosis was observed. After 36 weeks of the experiment, no hepatocellular carcinomas and no histopathological alterations were observed in the livers of the six rats in the control group (Table 5-1).

In a second experiment, groups of six male Wistar rats were exposed to 1000 ppm of dietary 2,4-toluenediamine for 8 or 20 weeks. There were no untreated controls. The rats were sacrificed 25 weeks after treatment began. These rats also exhibited poor growth. No hepatocellular tumors were observed in either group, as indicated in Table 5-1. Nodular hyperplasia was observed in both groups and occurred at a higher incidence in the group exposed to dietary 2,4-toluenediamine for 20 weeks than in the group exposed for 8 weeks (Ito et al., 1969).

TABLE 5-1

Incidence of Hepatocellular Carcinoma in Male Wistar Rats
Exposed to Dietary 2,4-Toluenediamine^{a,b}

Dose (ppm)	Duration of Treatment (weeks)	Duration of Study (weeks)	Tumor Incidence
0	36	36	0/6
600	34	34	7/11
1000	33	33	9/9
1000	8	24	0/6
1000	20	23	0/6

QUALITY OF EVIDENCE

Strengths of Study: Given in diet at two levels over a sufficient period of time for tumor development.

Weakness of Study: Only one sex; small number of animals; histological examination was limited to the liver. Treated rats had poor growth and early mortality; food consumption data were not reported.

Overall Adequacy: Adequate

^aSource: Ito et al., 1969

^bPurity of 2,4-toluenediamine was not reported.

Groups of 50 male and 50 female F344 rats were exposed to dietary levels of 125 or 250 ppm of 2,4-toluenediamine beginning at 6 weeks of age (NCI, 1979; Cardy, 1979; Sontag, 1981; Weisburger, 1983). Because of the resulting decreased body weight gain, however, the dose was lowered to 50 or 100 ppm, respectively, after 40 weeks of administration (NCI, 1979). The TWA concentration was 79 ppm in the low-dose group, given to both males and females for 103 weeks, 176 ppm, given to the high-dose males for 79 weeks and 171 ppm, given to the high-dose females for 84 weeks. Groups of 20 male and 20 female F344 rats served as controls. Gross and comprehensive histological examinations were performed on all rats that died, that were killed when moribund or that were killed at the end of the study, unless precluded by autolysis or cannibalism. There was a significant dose-related trend ($p < 0.001$) for increased mortality in both males and females. Survival at week 78 was 64% for high-dose males, 84% for low-dose males, 90% for control males, 92% for both treated female groups and 100% for control females. There was a dose-related and significant increase in the incidence of subcutaneous fibromas and hepatocellular carcinomas in male rats, as indicated in Table 5-2. The number of weeks to the first observed liver tumor appeared to be dose-related (76 weeks at the low dose; 57 weeks at the high dose).

Although there was a positive dose-related trend in the incidence of hepatocellular carcinoma/neoplastic nodules ($p = 0.008$, Cochran-Armitage) in female rats, there was no significant difference between the incidence of liver carcinomas/neoplastic lesions in either of the treated groups of female rats and the control group of female rats. There was a dose-related and significant increase in the incidence of subcutaneous fibromas in the high-dose group (Table 5-3). The incidence of mammary gland carcinoma or adenoma was significantly increased in both the low- and high-dose group.

TABLE 5-2

Incidence of Tumors in Male F344 Rats Exposed to Dietary 2,4-Toluenediamine (>99.9% pure) for 2 Years^a

TWA Concentration (ppm)	Duration of Treatment (weeks)	Duration of Study (weeks)	Target Organ	Tumor Type	Tumor Incidence ^b (p value)
0	104	104	subcutaneous tissue	fibroma	1/20 (0.009) ^c
79	103	103	subcutaneous tissue	fibroma	15/50 (0.020) ^d
176	79	79	subcutaneous tissue	fibroma	19/50 (0.004) ^d
0	104	104	liver	hepatocellular carcinoma or neoplastic nodule	0/20 (0.014) ^c
79	103	103	liver	hepatocellular carcinoma or neoplastic nodule	5/49 (NS) ^d
176	79	79	liver	hepatocellular carcinoma or neoplastic nodule	10/50 (0.026) ^d

QUALITY OF EVIDENCE

Strengths of Study: Sufficient duration and number of animals; relevant route of exposure; comprehensive histological examination; appropriate statistical methods; two dose groups; both sexes of two species.

Weakness of Study: High mortality rate in high dose group

Overall Adequacy: Adequate

^aSource: NCI, 1979

^bTumor incidence expressed as number of tumor-bearing rats/number of rats examined for that tumor type

^cCochran-Armitage test for dose-related trend

^dFisher's Exact test

NS = Not significant

TABLE 5-3

Incidence of Tumors in Female F344 Rats Exposed to Dietary 2,4-Toluenediamine (>99.9% pure) for 2 Years^a

TWA Concentration (ppm)	Duration of Treatment (weeks)	Duration of Study (weeks)	Target Organ	Tumor Type	Tumor Incidence (p value)
0	104	104	mammary gland	adenoma or carcinoma	1/20 (<0.001) ^b
79	103	103	mammary gland	adenoma or carcinoma	38/50 (<0.001) ^c
171	84	84	mammary gland	adenoma or carcinoma	41/50 (<0.001) ^c
0	104	104	subcutaneous tissue	fibroma	0/20 (p=0.009) ^b
79	103	103	subcutaneous tissue	fibroma	4/50 (NS)
171	84	84	subcutaneous tissue	fibroma	10/50 (0.026) ^c
<u>QUALITY OF EVIDENCE</u>					

Strengths of Study: See Table 5-2.

Weakness of Study: Increased mortality rate resulted in a reduced experimental duration

Overall Adequacy: Adequate

^aSource: NCI, 1979^bCochran-Armitage test for dose-related trend^cFisher's Exact test

NS = Not significant

The time to first observed mammary carcinoma or adenoma was shorter in the low dose (40 weeks) than in the high-dose females (76 weeks).

Other tumors occurring at increased incidences that were not significantly different from control, but that NCI (1979) considered to be possibly treatment-related were lung tumors, squamous-cell carcinomas of the skin and preputial gland, pancreatic acinar-cell adenomas in both males and females and mesotheliomas in males.

Groups of 50 male and 50 female B6C3F1 mice were exposed to dietary levels of 100 or 200 ppm of 2,5-toluenediamine for 101 weeks (Reuber, 1979; Sontag, 1981; Weisburger, 1983; NCI, 1979). The control group consisted of 20 male and 20 female B6C3F1 mice. There were no significant differences in survival; 75-90% of all groups lived until the end of the study and sufficient numbers of mice were at risk for late-developing tumors. Gross and comprehensive histological examinations were performed as previously described for rats. As indicated in Table 5-4, the only kind of tumor that occurred with significantly increased incidence after 2,4-toluenediamine exposure was hepatocellular carcinoma, observed in female mice. Although there was an increased incidence of hepatocellular carcinoma in male mice, the increase was not statistically significant. There was a significantly increased incidence of lymphoma/leukemia in low-dose females compared with controls ($p=0.001$) but not in high-dose females (see Table 5-4). Although the Cochran-Armitage test showed no positive dose-related trend, NCI (1979) considered the incidence of lymphoma/leukemia in low-dose female mice to be suggestive of a treatment-related carcinogenic effect. The incidence of alveolar/bronchiolar carcinoma in low-dose male mice was significantly increased above controls when analyzed by the Fisher Exact test; however, the p value of 0.039 is higher than the 0.025 level required by the

TABLE 5-4

Incidence of Tumors in B6C3F1 Mice Exposed to Dietary 2,4-Toluenediamine (99.9% pure) for 101 Weeks^{a,b}

Sex	Concentration (ppm)	Target Organ	Tumor Type	Tumor Incidence ^c (p value)
Female	0	liver	hepatocellular carcinoma	0/19 (0.002) ^d
	100	liver	hepatocellular carcinoma	13/47 (0.007) ^e
	200	liver	hepatocellular carcinoma	18/46 (0.001) ^e
Male	0	liver	hepatocellular carcinoma	5/20 (NS) ^d
	100	liver	hepatocellular carcinoma	17/50 (NS) ^e
	200	liver	hepatocellular carcinoma	13/49 (NS) ^e
Female	0	hematopoietic system	lymphoma/leukemia	2/19 (NS) ^d
	100	hematopoietic system	lymphoma/leukemia	29/47 (0.001) ^e
	200	hematopoietic system	lymphoma/leukemia	11/46 (NS) ^e
Male	0	lung	alveolar/bronchiolar carcinoma	0/20 (NS) ^d
	100	lung	alveolar/bronchiolar carcinoma	9/50 (0.039) ^e
	200	lung	alveolar/bronchiolar carcinoma	6/49 (NS) ^e

TABLE 5-4 (cont.)

Sex	Concentration (ppm)	Target Organ	Tumor Type	Tumor Incidence ^c (p value)
<u>QUALITY OF EVIDENCE</u>				
Strengths of Study: See Table 5-2				
Weakness of Study: None				
Overall Adequacy: Adequate				

^aSource: NCI, 1979

^bThe duration of the treatment was equal to the duration of the experiment

^cTumor incidence expressed as number of tumor-bearing mice/number of mice examined for that tumor type

^dCochran-Armitage test for dose-related trend

^eFisher Exact test

NS = Not significant

Bonferroni inequality criterion. This criterion requires that the p value for any comparison be ≤ 0.05 divided by number of dose groups. Furthermore, the Cochran-Armitage test for positive dose-related trend was not significant. NCI (1979), therefore, did not consider the lung tumors in male mice to be treatment-related.

Groups of 25 male and 25 female CD-1 HaM/ICR mice were exposed to 2,4-toluenediamine-2 HCl at dietary levels of 0, 500 or 1000 for 18 months (Weisburger et al., 1978). Mice were sacrificed 3 months after completion of treatment. Complete gross necropsy and histological evaluation of lung, liver, spleen, kidney, adrenal, heart, bladder, stomach, intestines and reproductive organs were performed. There was a significantly increased incidence of hepatomas in the female mice, but not in the male mice, in the high-dose group compared with the pooled control group only. There was a significantly increased incidence of vascular tumors in the females in the low-dose group and in the males in the high-dose group compared with the pooled control group only (Table 5-5).

Groups of 25 male Charles River CD rats were exposed to 2,4-toluenediamine-2 HCl at dietary levels of 0, 500 or 1000 ppm for 4 months, followed by dietary levels of 0, 250 or 500 ppm, respectively, for 14 months [TWA dose = 0, 306 or 611 ppm, respectively (Weisburger et al., 1978)]. The rats were sacrificed 6 months after treatment ended. There was a dose-related increase in the incidence of subcutaneous fibromas, which was significantly higher in both the low- and the high-dose groups (Table 5-6). A significant increase in the incidence of hepatomas occurred in the high-dose group when compared with the incidence of hepatomas in the pooled control group (see Table 5-6).

TABLE 5-5

Incidence of Tumors in Mice Exposed to Dietary 2,4-Toluenediamine·2 HCl
(97-99% purity) for 18 Months in a 21-Month Study^a

Sex	Concentration (ppm)	Target Organ	Tumor Type	Tumor Incidence (p value)
Female	0 (pooled)	liver	hepatoma	1/102
	0 (matched)	liver	hepatoma	0/22
	500	liver	hepatoma	1/19
	1000	liver	hepatoma	3/17 (<0.025) ^b
Female	0 (pooled)	vascular	NR	9/102
	0 (matched)	vascular	NR	4/22
	500	vascular	NR	5/19 (0.05-0.025) ^b
	1000	vascular	NR	4/17
Male	0 (pooled)	vascular	NR	5/99
	0 (matched)	vascular	NR	1/18
	500	vascular	NR	0/22
	1000	vascular	NR	4/17 (0.05-0.025) ^b

QUALITY OF EVIDENCE

Strengths of Study: Both sexes used; sufficient duration of exposure; two dose levels, relevant route of administration; extensive histological evaluation; appropriate statistical analysis

Overall Adequacy: Adequate

^aSource: Weisburger et al., 1978

^bFisher Exact test between treated and pooled controls

NR = Not reported

TABLE 5-6

Incidence of Tumors in Male Rats Exposed to Dietary
2,4-Toluenediamine·2 HCl (97-99% purity)
for 18 Months in a 24-Month Study^a

TWA Concentration (ppm)	Target Organ	Tumor Type	Tumor Incidence (p value)
0 (pooled)	subcutaneous tissue	fibroma	14/111
0 (matched)	subcutaneous tissue	fibroma	0/24
306	subcutaneous tissue	fibroma	8/19 (<0.025) ^b
611	subcutaneous tissue	fibroma	15/24 (<0.025) ^b
0 (pooled)	liver	hepatoma	2/11
0 (matched)	liver	hepatoma	0/24
306	liver	hepatoma	1/19
611	liver	hepatoma	3/24 (0.05-0.025) ^c

Quality of Evidence

Strengths of Study: Sufficient duration of treatment; relevant route; two dose levels; extensive histological examination; appropriate statistical tests

Weakness of Study: Only one sex

Overall Adequacy: Adequate

^aSource: Welsburger et al., 1978

^bFisher Exact test between treated and all controls

^cFisher Exact test between treated and pooled controls only

Groups of 36 male and 36 female Charles River rats were exposed to dietary 2,4-toluenediamine at a dose level of 1000 ppm for 2 weeks, followed by 500 ppm for 5.5 months and followed by 250 ppm for 9 months [TWA = 367 ppm (Du Pont, 1974)]. The rats were 30 days old at the start of treatment and were sacrificed at the end of the treatment period. There was a striking decrease in body weight gain of both sexes of treated rats and increased mortality in treated males compared with controls. Histological evaluation of the major organs and all gross lesions indicated that there was a statistically significant increased incidence of liver and mammary tumors in both male and female rats (Table 5-7) and of lung tumors in male rats.

Increased incidences of mammary tumors were also observed in female Sprague-Dawley rats (45 days of age) that were treated by gavage with 2,4-toluenediamine at 300 mg/kg/treatment, 3 times during a 30-day period or at 100 mg/kg/treatment, 10 times during a 30-day period and observed for an additional 9 months (Du Pont, 1974). There were 20 rats in the acetone-corn oil control group, 9 rats in the group treated 3 times and 22 rats in the group treated 10 times. Only the mammary gland was examined histologically. The incidences of mammary tumors (not otherwise specified) were 0/20 in the controls, 4/9 in the group treated 3 times ($p < 0.05$) and 5/22 in the group treated 10 times ($p < 0.05$). In a Russian study (Pylev et al., 1979) using female rats treated with chronic subcutaneous administration of 1-8 mg/kg of 2,4-toluenediamine, there was an increased incidence of mammary tumors and leukemia, which did not occur in female rats that received oral doses of 4 mg/kg of 2,4-toluenediamine 1-3 times/week for 16 months.

TABLE 5-7

Incidence of Tumors in Charles River Rats Treated for 15 Months in a 15-Month Study^a

Sex	TWA Concentration (ppm)	Target Organ	Tumor Type	Tumor Incidence (p value) ^b
Female	0	mammary gland	fibroadenoma	1/36
	367	mammary gland	fibroadenoma	16/36 (<0.025)
Female	0	mammary gland	adenofibroma	0/36
	367	mammary gland	adenofibroma	12/36 (<0.025)
Female	0	liver	neoplastic nodule	0/36
	367	liver	neoplastic nodule	21/36 (<0.025)
Male	0	liver	neoplastic nodule	0/36
	367	liver	neoplastic nodule	4/36 (NS)
Male	0	liver	hepatocellular carcinoma	0/36
	367	liver	hepatocellular carcinoma	11/36 (<0.025)
Male	0	lung	adenoma/adenocarcinoma	0/36
	367	lung	adenoma/adenocarcinoma	6/36 (<0.025)

TABLE 5-7 (cont.)

Sex	TWA Concentration (ppm)	Target Organ	Tumor Type	Tumor Incidence (p value) ^b
Male	0	mammary gland	fibroma/carcinofibrosarcoma	0/36
	367	mammary gland	fibroma/carcinofibrosarcoma	9/36 (<0.025)
<u>Quality of Evidence</u>				
Strengths of Study:		Both sexes; sufficient duration of exposure; extensive histological examination; appropriate statistical test		
Weaknesses of Study:		Only one dose level; dose levels changed with time during study		
Overall Adequacy:		Adequate		

^aSource: Du Pont, 1974

^bFisher Exact test

NS = Not significant

5.2. MUTAGENICITY

2,4-Toluenediamine is a potent mutagen in Salmonella typhimurium strains TA1538 and TA98 (Ames et al., 1975; Haworth et al., 1983; Shah et al., 1977; NRC, 1981) after metabolic activation. Without metabolic activation, it is a very weak mutagen for bacteria (Mori et al., 1982). The negative results reported in Neurospora crassa by Ong (1978) may have been due to the absence of a mammalian metabolic activation system. 2,4-Toluenediamine was mutagenic in Drosophila melanogaster as measured by the induction of recessive lethal mutations (Fahmy and Fahmy, 1977; Blijleven, 1977). The micronucleus test, evaluating polychromatic erythrocytes in five male and five female rats 6 hours after an almost lethal gavage dose, was negative (Hossack and Richardson, 1977); however, the sampling time is too short for a definitive study. There was a statistically significant increase in the number of chromatid breaks in women who dyed their hair when compared with age-matched controls (women who did not dye their hair) (Kirkland et al., 1978). This result is inconclusive because in addition to 2,4-toluenediamine there are many other components in hair dye that are capable of causing chromatid breaks and because no control of variables (in addition to hair-dyeing), such as smoking, was reported.

2,4-Toluenediamine was mutagenic at the thymidine kinase locus in mouse lymphoma L5178Y cells without metabolic activation and mutagenic to Chinese hamster ovary AT3-2 cells both with and without metabolic activation (Coppinger et al., 1984); however, negative results were observed at the hgpri locus in both cell types. In the presence of 2,4-toluenediamine hamster embryo cells were morphologically transformed in vitro without exogenous metabolic activation (Pienta and Kawalek, 1981). 2,4-Toluenediamine enhanced the viral transformation of hamster embryo cells when

administered after viral inoculation but not when administered before viral inoculation (Greene and Friedman, 1980), suggesting the possibility that the active metabolite is short-lived.

Groups of 20 male DBA/2J mice were treated either orally or intraperitoneally with 0 or 80 mg/kg of 2,4-toluenediamine on 2 consecutive days (40 mg/kg/day) (Soares and Lock, 1980). Each male mouse was mated with three females/week during the following 7 weeks. There were no significant effects on the number of implants/pregnant female, the mean percent of postimplantation deaths/pregnant female or on the percent of fertile matings based on the total number of paired females. A dominant lethal assay by Burnett et al. (1977), in which a group of 20 male Charles River CD rats was injected intraperitoneally with 20 mg/kg of 2,4-toluenediamine 3 times/week for 8 weeks and then mated with two females each for 1 week, reported no significant effects on the mean number of implants, live fetuses and resorptions/pregnancy. A group of 40 male rats that received intraperitoneal injections of sterile water served as a control group. These results indicate that 2,4-toluenediamine did not induce dominant lethals in mice within the parameters of these studies. However, the use of a single dose level in each of these studies precludes a definite conclusion.

The synthesis of DNA in mouse testes, however, was significantly depressed 3.5 hours after groups of five C57B/6XC3H male mice were injected intraperitoneally with 40.0, 44.5, 49.5 or 55.0 mg/kg of 2,4-toluenediamine (Greene et al., 1981).

No morphological abnormalities were observed in caudal sperm collected 5 weeks after groups of five male (CBA BALB/c)F1 mice were treated intraperitoneally with 50 or 100 mg/kg/day of 2,4-toluenediamine for 5 consecutive days (Topham, 1980). A group of five male mice was treated intraperitoneally with the vehicle only (Tween 80), and served as a control group.

5.3. TERATOGENICITY

Groups of 50 pregnant specific pathogen free CD-1 mice were treated by gavage with 0 or 150 mg/kg/day 2,4-toluenediamine in corn oil on days 7-14 of pregnancy (Smith, 1983). Body weights of dams and pups were recorded. All mice were terminated 23 days after mating. There was a statistically significant decrease in the reproductive index and the number of viable litters, as well as a significant increase in the number of resorptions, maternal deaths and stillbirths in the mice treated with 2,4-toluenediamine. The data presented by Smith (1983) are summarized in Table 5-8.

5.4. OTHER REPRODUCTIVE EFFECTS

The health effects of occupational exposure to 2,4-toluenediamine and dinitrotoluene were first investigated due to a perceived increase in the rate of spontaneous abortions by the wives of exposed men at a toluene diisocyanate and polyurethane plastics plant. In a study in Brandenburg, KY, no significant reproductive effects, including sperm counts and spontaneous abortions, were reported (Olin Corp., 1980). Exposure levels ranged from not detectable to 0.39 mg/m³ for an operator exposure (work area values ranged between not detectable levels to 0.038 mg/m³). Although not statistically significant, there was a dose-related decrease in sperm count in men exposed to 2,4-toluenediamine; however, the lack of statistical significance may be attributed to the large variance in the sperm count and the small number of workers evaluated (Table 5-9). In addition, there was an increased incidence of miscarriages in the wives of workers exposed to 2,4-toluenediamine and dinitrotoluene, but the increase was not statistically significant. Although the Olin Corp. (1980) reported that the differences in sperm count were not significant, another source reported that the

TABLE 5-8

Summary of Fetal Developmental and Maternal Toxicity Data^a

Parameter	Vehicle Control (corn oil)	Test Dose (150 mg/kg)	Statistical Analysis ANOVA
Number of viable litters (n)	30	5	NR
Number of stillborn litters	1	4	NR
Number of live pups/litter within 12 hours postpartum	10.6 $\pm$ 2.4	5.4 $\pm$ 2.7	α <0.01
Number of dead pups/litter within 12 hours postpartum	0.03 $\pm$ 0.18	0.80 $\pm$ 1.10	α <0.01
Number of Live pups/litter day 3 postpartum	10.0 $\pm$ 2.8	4.4 $\pm$ 2.9	α <0.01
Number mice mated	50	50	NA
Number died during test:			
Dosing period	0	4	NR
Post-dosing	0	13	NR
Non-pregnant	0	1	NR
Number mated mice with totally resorbed litters ^b	2	24 ^c	NR
Number not pregnant	16	14	NR
Reproduction index ^d	0.91	0.14	α <0.01

^aSource: Smith, 1983^bAs determined by the sulfide nidation site test^cIncluding the 13 mice that died^dRatio of the number of animals producing viable litters divided by the number of mice ever pregnant

NA = Not applicable; NR = not reported

TABLE 5-9

Semen Volumes and Sperm Counts from Three Groups of Workers
Occupationally Exposed to 2,4-Toluenediamine*

Level of Exposure	Number Evaluated	Semen Volume (cc)		Sperm Count	
		Mean	Standard Deviation	Mean	Standard Deviation
Control	9	2.5	± 1.4	156.8×10^6	± 111.8
Intermediate	12	3.0	± 1.8	121.3×10^6	± 95.1
High	9	2.8	± 2.1	55.9×10^6	± 47.3

*Source: Olin Corp., 1980

sperm counts were significantly reduced in workers exposed to the toluene diisocyanate precursors, 2,4-toluenediamine and dinitrotoluene (Anonymous, 1981).

In a subsequent study of a larger number of workers (203) at a toluene diisocyanate and polyurethane plastics plant in Charles Lake, LA, no significant reproductive effects were observed after evaluation of each worker by a urogenital examination, a reproductive history questionnaire, determination of serum follicle-stimulating hormone and a sperm count (Hamill et al., 1982). Exposure levels were comparable to the Brandenburg, KY site. Non-reproductive parameters were not evaluated.

5.5. CHRONIC AND SUBCHRONIC TOXICITY

In the Du Pont (1974) study (see Section 5.1.), in which groups of 36 male and 36 female Charles River CD rats were exposed to dietary 2,4-toluenediamine at a level of 1000 ppm for 2 weeks, followed by 500 ppm for 5.5 months and followed by 250 ppm for 9 months, histological evaluation of the major organs, blood chemistry and hematology, body weights and mortality rates were reported. A group of 36 male and 36 female CD rats served as controls. There was a significant decrease in the body weight gain of males and females and a significantly increased mortality rate in the males. The males had an increased volume of dilute urine, inflammation of the renal pelvis, proteinuria and glycosuria, while both sexes had an increased level of urinary urobilinogen. Although the effects were greater in the males than in the females, both sexes had increased levels of serum alkaline phosphatase, SGPT and bilirubin accompanied by histological alteration of the liver. Severe splenic atrophy and increased hemosiderin levels were reported in both sexes. In the males, severe testicular atrophy and granuloma formation occurred. An increased incidence of liver and mammary tumors in both sexes and lung tumors in males was reported (see Section 5.1.).

In the NCI (1979) bioassay in which groups of 50 male and 50 female F344 rats were exposed to a TWA dose of 79 ppm (both sexes) or 171 ppm (females) and 179 ppm (males) for up to 103 weeks, there was a significant dose-related decrease in body weight gain and survival rate; the high-dose groups were terminated after 79 weeks for the males and after 84 weeks for the females. A group of 20 male and 20 female rats served as controls. Although chronic renal disease is usually present in aging F344 rats, renal disease in the rats exposed to dietary 2,4-toluenediamine was appreciably more severe and occurred at an earlier age than in the control rats. In treated male rats, high incidences of hyperparathyroidism, secondary to renal disease, were also observed. Histopathological lesions in the liver of rats of both sexes and dose groups were observed at increased incidences and ranged from focal fatty changes to severe diffuse degeneration.

In the Ito et al. (1969) study in which groups of 12 male Wistar rats were exposed to 600 or 1000 ppm of dietary 2,4-toluenediamine for 33-34 weeks, treated rats had dose-related marked decreased body weight gain; after 4 weeks they stopped gaining weight and after 8 weeks they gradually lost weight until they died or were killed. Food consumption data were not reported. The control group consisted of six male Wistar rats fed diets not contaminated with 2,4-toluenediamine. Fatty changes and cholangiofibrosis were seen in the livers of rats exposed to both 600 and 1000 ppm 2,4-toluenediamine, but minimal cirrhosis occurred only at 1000 ppm. Nonhepatic effects of 2,4-toluenediamine were not evaluated.

In groups of 50 male and 50 female B6C3F1 mice exposed to dietary levels of 100 or 200 ppm of 2,4-toluenediamine for 101 weeks, mean body weights were decreased compared with controls (20 male and 20 female B6C3F1 mice)

except in the low-dose male group (NCI, 1979). Survival rate of the mice was unaffected. Normal liver histology was altered in both male and female mice at both dose levels (Table 5-10).

The occupational study of workers exposed to 2,4-toluenediamine in the Brandenburg, KY, plastics plant (see Section 5.4.) reported no significant reproductive effects in the 30 workers evaluated (Olin Corp., 1980). The workers were divided into three groups: nine unexposed controls; 12 exposed to an intermediate level of 2,4-toluenediamine; and nine exposed to a high level. Although the levels of exposure were not reported for each group, the highest level of 2,4-toluenediamine measured in the plant was 0.39 mg/m³. The SGOT and SGPT levels of the workers in the high exposure group were higher than those of the workers in the low-dose and control groups, but the increase was not statistically significant.

5.6. OTHER RELEVANT INFORMATION

The acute oral toxicity (LD₅₀) values for 2,4-toluenediamine are 179-212 mg/kg for male rats (Dow Chemical Co., 1983; Air Products and Chemicals, Inc., 1973), 380 mg/kg in male mice (Dow Chemical Co., 1983) and ~500 mg/kg for rabbits (Dow Chemical Co., 1983).

5.7. SUMMARY

2,4-Toluenediamine was carcinogenic following dietary administration to mice and rats at levels ≥ 79 ppm, producing a significant increase in the incidence of a large variety of tumor types including liver (NCI, 1979; Ito et al., 1969; Weisburger et al., 1978; Du Pont, 1974), mammary gland (NCI, 1979; Du Pont, 1974), subcutaneous fibromas (NCI, 1979; Weisburger et al., 1978), lung (Du Pont, 1974), lymphomas (NCI, 1979; Weisburger et al., 1978) and leukemia (NCI, 1979). Liver tumors were the most prevalent tumor type and were observed in both rats and mice. The results of mutagenicity

TABLE 5-10

Incidence of Hepatic Hyperplasia in Male and Female
Mice Exposed to Dietary 2,4-Toluenediamine*

Dose (ppm)	Hyperplasia NOS	Hyperplasia Diffuse	Hyperplasia Nodular
MALE			
0	0/20	0/20	0/20
100	11/50	8/50	6/50
200	26/49	4/49	4/49
FEMALE			
0	0/20	0/20	0/20
100	8/47	13/47	9/47
200	5/46	0/46	22/46

*Source: NCI, 1979

NOS = Not otherwise specified

testing were strongly positive in bacterial assays only after metabolic activation (Ames et al., 1975; Haworth et al., 1983; Shah et al., 1977; NRC, 1981), but were inconclusive in mammalian in vitro assays. 2,4-Toluenediamine does not appear to cause dominant lethal mutations in mice (Soares and Lock, 1980) or rats (Burnett et al., 1977), but does depress DNA synthesis in the mouse testis (Greene et al., 1981). There was increased mortality and a significant decrease in the number of viable litters in mice treated orally with 2,4-toluenediamine at 150 mg/kg during pregnancy (Smith, 1983). Although not statistically significant, there was a decreased sperm count in the workers occupationally exposed to 2,4-toluenediamine, and an increase in the spontaneous abortion rates of the wives of 2,4-toluenediamine-exposed workers (Olin Corp., 1980). In a larger follow-up study, no adverse reproductive effects of 2,4-toluenediamine exposure were reported (Hamill et al., 1982). After chronic dietary exposure to ≥ 79 ppm, 2,4-toluenediamine was hepatotoxic to rats (Du Pont, 1974; Ito et al., 1969; Welsburger et al., 1978; NCI, 1979) and mice (NCI, 1979). 2,4-Toluenediamine was nephrotoxic to rats at dietary levels ≥ 79 ppm (Du Pont, 1974; NCI, 1979). Increased levels of BUN and increased serum levels of liver enzymes were observed in men occupationally exposed, but the differences from control levels were not statistically significant (Olin Corp., 1980). Dietary administration of 2,4-toluenediamine to rats and mice resulted in striking dose-related decreases in growth rates beginning early in the treatment schedule (NCI, 1979; Ito et al., 1969; DuPont, 1974). Because no food consumption data were reported in these studies, it is not possible to determine if the poor growth rates were due to the toxicity of the chemical or to food refusal.

6. AQUATIC TOXICITY

6.1. ACUTE

Exposure to 5 mg/l 2,4-toluenediamine for 24 hours had no effect on rainbow trout, Salmo gairdneri, bluegills, Lepomis macrochirus, or larval sea lampreys, Petromyzon marinus (Applegate et al., 1957). Smirnova et al. (1967) reported that Daphnia died 5-10 days after exposure to 5 mg/l of 2,3-toluenediamine.

6.2. CHRONIC

Pertinent data regarding the chronic toxicity of 2,4-toluenediamine to aquatic organisms could not be located in the available literature as cited in the Appendix.

6.3. PLANT

Pertinent data regarding the chronic toxicity of 2,4-toluenediamine to aquatic plants could not be located in the available literature as cited in the Appendix.

6.4. RESIDUES

Veith et al. (1979) determined a BCF of 91 for an unspecified isomer of toluediamine in fathead minnows, Pimephales promelas, in a 32-day flow-through exposure at 25°C.

6.5. SUMMARY

Data regarding the aquatic toxicity of 2,4-toluenediamine are limited. The BCF for the unspecified isomer of toluediamine was reported to be 91 in fathead minnows (Veith et al., 1979).

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

Pertinent guidelines and standards, including EPA ambient water and air quality criteria, drinking water standards, FAO/WHO ADIs, EPA or FDA tolerances for raw agricultural commodities or foods, and ACGIH, NIOSH or OSHA occupational exposure limits could not be located in the available literature as cited in the Appendix.

7.2. AQUATIC

Guidelines and standards for the protection of aquatic organisms from the toxic effects of 2,4-toluenediamine could not be located in the available literature as cited in the Appendix.

8. RISK ASSESSMENT

Rats and mice exposed orally to 2,4-toluenediamine develop a variety of tumors. According to the NCI (1979) bioassay, dietary exposure of rats to TWA levels ≥ 79 ppm and of mice to ≥ 100 ppm was associated with an increased incidence of subcutaneous fibromas in male and female rats, an increased incidence of mammary tumors (adenoma or carcinoma) in female rats, an increased incidence of liver tumors in male rats and an increased incidence of liver tumors in female mice. Although there was an increased incidence of liver tumors in male mice, the increase was not statistically significant. At higher dietary levels (>300 ppm), 2,4-toluenediamine caused liver tumors in male and female rats and mice (Ito et al., 1969; Weisburger et al., 1978; Du Pont, 1974). Although these studies used higher doses of 2,4-toluenediamine, the rats and mice were dosed for a shorter period (≤ 18 months) than in the NCI (1979) bioassay and some rats and mice were observed for up to 6 months after termination of treatment [Weisburger et al., 1978 (see Section 5.1.)]. In addition to liver tumors, several other tumors were observed. There was an increased incidence of subcutaneous fibromas in male rats at TWA levels ≥ 306 ppm (only males were studied) (Weisburger et al., 1978), an increased incidence of lung tumors in male rats at a TWA level of 367 ppm (Du Pont, 1974) and an increased incidence of mammary tumors in female and male rats (Du Pont, 1974). Increased incidences of mammary tumors were also observed in female rats treated by gavage at 300 mg/kg/treatment, 3 times during a 30-day period or at 100 mg/kg/treatment, 10 times during a 30-day period and observed for an additional 9 months (Du Pont, 1974).

2,4-Toluenediamine was a potent mutagen in bacterial assays after metabolic activation (Ames et al., 1975; Haworth et al., 1983; Shah et al., 1977; NRC, 1981) and caused recessive lethal mutations in D. melanogaster (Fahmy and Fahmy, 1977; Blijleven, 1977). Dominant lethal mutations were not increased in mice treated orally or intraperitoneally with 2,4-toluenediamine (Soares and Lock, 1980), but testicular DNA synthesis was significantly depressed in mice treated intraperitoneally with 2,4-toluenediamine (Greene et al., 1981). Dominant lethal mutations are not increased in rats after intraperitoneal injection of 2,4-toluenediamine (Burnett et al., 1977).

No teratogenic effects were reported in the offspring of mice treated orally with 150 mg/kg/day of 2,4-toluenediamine during pregnancy, but there were severe fetotoxicity and high mortality of dams at the dose level tested (Smith, 1983). According to Smith (1983), there was a significant increase in the number of totally resorbed litters and a decrease in the number of viable litters.

Although no statistically significant adverse reproductive effects were associated with occupational exposure (Olin Corp., 1980; Hamill et al., 1982), the sperm count was depressed in workers occupationally exposed to the highest level (≤ 0.39 mg/m³) of 2,4-toluenediamine at the Brandenburg, KY, plant (Olin Corp., 1980; Anonymous, 1981). In a subsequent study at a plant in Lake Charles, LA, which provided a larger sample size, no effect on sperm counts was reported at any exposure level; exposure levels were comparable to the Brandenburg, KY site (Hamill et al., 1982).

After chronic dietary exposure, 2,4-toluenediamine is hepatotoxic in rats (Du Pont, 1974; Ito et al., 1969; Weisburger et al., 1978; NCI, 1979) and mice (NCI, 1979). The lowest dose levels at which liver toxicity was observed in rats and mice were reported in the NCI (1979) bioassay; rats exposed to TWA dietary levels ≥ 79 ppm had focal degenerative liver changes

and male and female mice exposed to dietary levels ≥ 100 ppm had significantly increased incidences of liver hyperplasia and focal histological alterations. In humans exposed occupationally to 2,4-toluenediamine, the highest exposure level was reported to be 0.39 mg/m^3 at the Brandenburg, KY, plant. There was an increase in SGOT and SGPT levels of the workers in the highest exposure groups; however, the increases were not statistically significant compared with controls. Despite the lack of statistical significance, these results in conjunction with the results of the animal experiments suggest that 2,4-toluenediamine is hepatotoxic in humans.

2,4-Toluenediamine appears to be nephrotoxic in rats at dietary levels ≥ 79 ppm (Du Pont, 1974; NCI, 1979), but not mice at dietary levels ≥ 200 ppm (NCI, 1979). The human data indicate a slight, not statistically significant increase in BUN levels in workers occupationally exposed to 2,4-toluenediamine.

The carcinogenicity of 2,4-toluenediamine warrants the derivation of a q_1^* rather than an ADI. Because of the shortcomings of the Ito et al. (1969) study, the Weisburger et al. (1978) study (which used only male rats) and the Du Pont (1974) study which used only one dose level, the NCI (1979) bioassay is the most appropriate study from which to derive a q_1^* . In the NCI (1979) bioassay (Tables 8-1 to 8-5), the male rats exposed to the high dose of 2,4-toluenediamine were terminated at 79 weeks and the female rats at 84 weeks after treatment began. Therefore, the correction factors for interspecies extrapolation (the cube root of the body weight ratios) and the less than lifetime exposure (the ratio of length of experiment to lifespan cubed) were applied to the dose levels before using the multistage model, GLOBAL 82, developed by Howe and Crump (1982). The appropriate methodology for these correction factors which has been discussed in U.S. EPA (1980) is presented as follows.

TABLE 8-1
Cancer Data Sheet for Derivation of q_1^*

Compound: 2,4-toluenediamine

Reference: NCI, 1979

Species, Strain, Sex: mice, B6C3F1, female

Body weight (kg): 0.047 (control); 0.037 (low dose); 0.029 (high dose)

Length of exposure (t_e) = 101 weeks

Length of experiment (L_e) = 101 weeks

Lifespan of animal (L) = 104 weeks

Tumor site and type: hepatocellular

Route, vehicle: oral, dietary

Experimental Doses (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/ No. Examined
0	0	0/19
100	$100 \times 0.13 \times (0.037/70)^{1/3} \times (101/104)^2 = 0.96$	13/47
200	$200 \times 0.13 \times (0.029/70)^{1/3} \times (101/104)^2 = 1.78$	18/46

Unadjusted q_1^* from study (not derived, see text)

Human $q_1^* = 3.9987 \times 10^{-1} \text{ (mg/kg/day)}^{-1}$

TABLE 8-2
Cancer Data Sheet for Derivation of q_1^*

Compound: 2,4-toluenediamine

Reference: NCI, 1979

Species, Strain, Sex: rats, F344, male

Body weight (kg): 0.43 (controls); 0.35 (low dose); 0.28 (high dose)

Length of exposure (t_e) = 103 weeks (low dose); 79 weeks (high dose)

Length of experiment (L_e) = 103 weeks (low dose); 79 weeks (high dose)

Lifespan of animal (L) = 104 weeks

Tumor site and type: subcutaneous fibroma

Route, vehicle: oral, dietary

Experimental Doses (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/ No. Examined
0	0	1/20
79	$79 \times 0.05 \times (0.35/70)^{1/3} \times (103/104)^3 = 0.66$	15/50
176	$176 \times 0.05 \times (0.28/70)^{1/3} \times (79/104)^3 = 0.61$	19/50

Unadjusted q_1^* from study (not derived, see Text)

Human $q_1^* = 7.9939 \times 10^{-1} \text{ (mg/kg/day)}^{-1}$

TABLE 8-3

Cancer Data Sheet for Derivation of q_1^*

Compound: 2,4-toluenediamine

Reference: NCI, 1979

Species, Strain, Sex: rats, F344, male

Body weight (kg): 0.43 (control); 0.35 (low dose); 0.28 (high dose)

Length of exposure (t_e) = 103 weeks (low dose); 79 weeks (high dose)Length of experiment (L_e) = 103 weeks (low dose); 79 weeks (high dose)Lifespan of animal (L) = 104 weeks

Tumor site and type: hepatocellular carcinoma or neoplastic nodule

Route, vehicle: oral, dietary

Experimental Doses (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/ No. Examined
0	0	0/20
79	$79 \times 0.05 \times (0.35/70)^{1/3} \times (103/104)^3 = 0.66$	5/49
176	$176 \times 0.05 \times (0.28/70)^{1/3} \times (103/104)^3 = 0.61$	10/50

Unadjusted q_1^* from study (not derived, see text)Human $q_1^* = 3.8464 \times 10^{-1} \text{ (mg/kg/day)}^{-1}$

TABLE 8-4

Cancer Data Sheet for Derivation of q_1^*

Compound: 2,4-toluenediamine

Reference: NCI, 1979

Species, Strain, Sex: rats, F344, female

Body weight (kg): 0.275 (control); 0.220 (low dose); 0.175 (high dose)

Length of exposure (t_e) = 103 weeks (low dose); 84 weeks (high dose)Length of experiment (L_e) = 103 weeks (low dose); 84 weeks (high dose)Lifespan of animal (L) = 104 weeks

Tumor site and type: mammary gland (adenoma/carcinoma)

Route, vehicle: oral, dietary

Experimental Doses (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/ No. Examined
0	0	1/20
79	$79 \times 0.05 \times (0.22/70)^{1/3} \times (103/104)^3 = 0.56$	38/50
171	$171 \times 0.05 \times (0.175/70)^{1/3} \times (84/104)^3 = 0.61$	41/50

Unadjusted q_1^* from study (not derived, see text)Human $q_1^* = 3.1751 \text{ (mg/kg/day)}^{-1}$

TABLE 8-5

Cancer Data Sheet for Derivation of q_1^*

Compound: 2,4-toluenediamine

Reference: NCI, 1979

Species, Strain, Sex: rats, F344, female

Body weight (kg): 0.275 (control); 0.220 (low dose); 0.175 (high dose)

Length of exposure (t_e) = 103 weeks (low dose); 84 weeks (high dose)Length of experiment (t_e) = 103 weeks (low dose); 84 weeks (high dose)Lifespan of animal (L) = 104 weeks

Tumor site and type: subcutaneous/fibroma

Route, vehicle: oral, dietary

Experimental Doses (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/ No. Examined
0	0	0/20
79	$79 \times 0.05 \times (0.22/70)^{1/3} \times (103/104)^a = 0.56$	4/50
171	$171 \times 0.05 \times (0.175/70)^{1/3} \times (84/104)^a = 0.61$	10/50

Unadjusted q_1^* from study (not derived, see text)Human $q_1^* = 3.7553 \times 10^{-1} \text{ (mg/kg/day)}^{-1}$

As suggested by Mantel and Schneiderman (1975), we assume that mg/surface area/day is an equivalent dose between species. Since to a close approximation the surface area is proportional to the 2/3rds power of the weight as would be the case for a perfect sphere, the exposure in mg/2/3rds power of the body weight/day is similarly considered to be an equivalent exposure. In an animal experiment, this equivalent dose is computed in the following manner:

Let:

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e)

W = average weight of the experimental animal.

Then, the lifetime average exposure is

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

If the duration of experiment (L_e) is less than the natural lifespan of the test animal (L), the slope q_1^* , or more generally the exponent $g(d)$, is increased by multiplying a factor $(L/L_e)^3$. We assume that if the average dose, d , is continued, the age-specific rate of cancer will continue to increase as a constant function of the background rate. The age-specific rates for humans increase at least by the 2nd power of the age and often by a considerably higher power, as demonstrated by Doll (1971). Thus, we would expect the cumulative tumor rate to increase by at least the 3rd power of age. Using this fact, we assume that the slope q_1^* , or more generally, the exponent $g(d)$, would also increase by at least the 3rd power of age. As a result, if the slope q_1^* [or $g(d)$] is calculated at age L_e , we would expect that if the experiment had been continued for the full lifespan, L , at the given average exposure, the slope q_1^* [or $g(d)$] would have been increased by at least $(L/L_e)^3$.

This adjustment is conceptually consistent to the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Crump et al. (1977).

The dose levels used to derive the q_1^* for the various tumor types in the rats of the NCI (1979) bioassay were obtained in the following manner: the dose level in ppm was multiplied by the food factor for rats (0.05) to

obtain the dose in mg/kg/day; the dose in mg/kg/day was multiplied by the cube root of the ratio of the animal body weight (experimentally derived) to the reference human body weight (70 kg) and by the third power of the length of the experiment (79 weeks for high dose males, 84 weeks for high dose females, 103 weeks for low dose males and females) divided by the lifespan of the rats (104 weeks).

The dose levels used to derive the q_1^* for hepatocellular carcinoma in female mice, the only tumor type whose incidence is significantly increased in the mice, were obtained by multiplying the dose level in ppm by the food factor (0.13). Because the experimental body weights were different (controls, 0.047 kg; low dose, 0.037 kg; high dose, 0.029 kg), the correction factors for interspecies extrapolation (the cube root of the body weight ratios) and the less than lifetime exposure (the ratio of length of experiment to lifespan cubed) were applied to the dose levels before using the multistage model. As indicated in Table 8-6, the highest q_1^* of 3.2 (mg/kg/day)⁻¹ is obtained from the data on the incidence of mammary tumors in female rats.

In a previous risk assessment of 2,4-toluenediamine (SRC, 1982), a q_1^* of 4.03 (mg/kg/day)⁻¹ was derived based on the incidence of mammary tumors in female rats in the NCI (1979) study. The q_1^* derived in the present determination is slightly lower. The difference can be attributed in part to the use of a different duration of experiment: whereas SRC (1982) used the duration of the experiment for high-dose female rats (588 days or 84 weeks) for calculating the q_1^* for humans, the present determination uses the durations for the individual groups and corrects the dose for lifespan before using the computerized model. In addition, SRC (1982) may have used the reference body weight of rats (0.35 kg), which would contribute to the discrepancy.

TABLE 8-6
Values of q_1^* for Various Tumors*

Species	Sex	Tumor Location	Human q_1^* (mg/kg/day) ⁻¹
Mice	female	liver	4×10^{-1}
Rats	female	mammary gland	3.2
Rats	female	subcutaneous tissue	3.8×10^{-1}
Rats	male	subcutaneous tissue	8×10^{-1}
Rats	male	liver	3.8×10^{-1}

*Source: NCI, 1979

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

As summarized in Table 9-1 and discussed in detail in Sections 5.3. and 5.5., there are four studies (Smith, 1983; Du Pont, 1974; Ito et al., 1969; NCI, 1979) that report toxicity data in addition to carcinogenicity data. In the Smith (1983) study, groups of 50 pregnant CD-1 mice were treated by gavage with 0 or 150 mg/kg/day of 2,4-toluenediamine in corn oil on days 7-14 of pregnancy (Smith, 1983). In the Du Pont (1974) study, groups of 36 male and 36 female Charles River CD rats were exposed to dietary 2,4-toluenediamine at a level of 1000 ppm for 2 weeks, followed by 500 ppm for 5.5 months and followed by 250 ppm for 9 months (TWA = 367 ppm). The dietary exposure was converted to 18 mg/kg/day by multiplying by the food factor of 0.05 for rats (U.S. EPA, 1980). In the Ito et al. (1969) study, groups of 12 male Wistar rats were exposed to 600 or 1000 ppm of dietary 2,4-toluenediamine for 33-34 weeks. The dietary exposure was converted to 30 or 50 mg/kg/day, respectively, by multiplying by the food factor of 0.05 for rats. In the NCI (1979) bioassay, groups of 50 male and 50 female F344 rats were exposed to a TWA of 79 ppm (male and female) or 171 ppm (female) and 176 ppm (male) for up to 103 weeks in the diet and groups of 50 male and 50 female B6C3F1 mice were exposed to dietary levels of 100 or 200 ppm of 2,4-toluenediamine for 101 weeks. The dietary exposure was converted to 3.95 mg/kg/day for the low-dose rats, 8.55 mg/kg/day for the high-dose female rats, 8.8 mg/kg/day for the high-dose male rats, 13 mg/kg/day for the low-dose mice and 26 mg/kg/day for the high-dose mice by multiplying by the food factor of 0.05 or 0.13 for rats or mice, respectively (U.S. EPA, 1980).

TABLE 9-1

Toxicity Summary for 2,4-Toluenediamine

Route	Species/ Strain	Sex	Number at Start	Average Weight (kg)	Vehicle/ Physical State	Purity	Exposure	Transformed Animal Dose (mg/kg/day)	Response	Reference
Oral (gavage)	mice/CD-1	pregnant females	50	0.03	corn oil	NR	150 mg/kg/day on days 7-14 of gestation	150	Fetotoxic: decreased number of viable litters Maternotoxic: death	Smith, 1983
Oral	rats/CD	male and female	36/36	0.275 (males) 0.180 (females)	dietary	NR	1000 ppm for 2 weeks 500 ppm for 5.5 months 250 ppm for 9 months (TMA = 367 ppm)	18	Decreased body weight gain (male and females); in- creased mortality (males); increased urinary output; and inflammation of renal pelvis increased SGPT levels accompanied by hepatic histological changes; hemosiderosis; severe splenic and testic- ular atrophy	Du Pont, 1974
Oral	rats	male	12	0.255	dietary	NR	600 ppm for 34 weeks	50	Decreased body weight gain; fatty changes and cirrhosis in liver as well as increased tumor formation	Ito et al., 1969
				0.225			1000 ppm for 33 weeks	30	Fatty changes in the liver as well as increased tumor formation; decreased body weight gain	
Oral	rats	male and female	50/50	0.350 (males) 0.220 (females)	dietary	>99.9%	79 ppm for 103 weeks	3.95	Decreased body weight gain and decreased survival rate; hepatic histo- pathology; increased rate of kidney disease Same as above	MCI, 1979
				0.280			176 ppm for 79 weeks in males	8.8		
Oral	mice	male and female	50/50	0.175			171 ppm for 84 weeks in females	8.55	Same as above	
				0.041 (males) 0.037 (females)	dietary	>99.9%	100 ppm for 103 weeks	13	Decreased body weight gain; hepatic histo- pathology probably pre- neoplastic	MCI, 1979
				0.037 (males) 0.029 (females)			200 ppm for 103 weeks	26	Same as above	

NR = Not reported

The most severe effect of 2,4-toluenediamine dietary exposure observed in rats was increased mortality, which occurred at the lowest transformed animal dose (3.95 mg/kg/day) used in all the studies. The MED of 40.5 mg/day for increased mortality (Table 9-2) is calculated by multiplying 3.95 mg/kg/day by the cube root of the ratio of the average body weight of female rats (0.22 kg; see Table 9-1) in the NCI (1979) study to the reference human body weight of 70 kg (U.S. EPA, 1980), and by 70 kg to express the MED in mg/day. The MED corresponds to an RV_d of 3.1. Increased mortality warrants an RV_e of 10. Multiplying the RV_d by the RV_e results in a CS of 31, which corresponds to an RQ of 100.

In mice, diffuse to nodular hyperplasia occurred in male and female mice at both doses; however, the hyperplasia could be considered to be preneoplastic, as increased incidences of hepatocellular carcinoma/adenoma occurred in female mice (NCI, 1979). Thus, it is not appropriate to calculate a CS for liver histopathology. There was also a dose-related decrease in body weight gain of female mice; decreased body weight gain was observed in male mice only at the high dose (NCI, 1979). The MED of 73.6 mg/day for decreased body weight gain of mice was calculated using the average body weight of low-dose female mice (0.037 kg; see Table 9-1) in the NCI (1979) study. The MED corresponds to an RV_d of 2.7. The RV_e for decreased body weight gain is 4, the CS is 10.8, corresponding to an RQ of 1000 (see Table 9-2).

Decreased numbers of viable litters occurred in mice at 150 mg/kg/day (Smith, 1983). The MED of 792 mg/day was calculated using the average body weight of nongravid mice (0.03 kg; see Table 9-1). The RV_d is 1.2, the RV_e is 8 and the CS is 9.6, corresponding to an RQ of 1000.

TABLE 9-2
Oral Composite Scores for 2,4-Toluenediamine

Species	Animal Dose (mg/kg/day)	Chronic Human MED (mg/day)	RV _d	Effect	RV _e	CS	RQ	Reference
Mouse	150	791	1.2	Maternal and fetal toxicity	8	9.6	1000	Smith, 1983
Mouse	13	73.6	2.7	Decreased body weight gain	4	10.8	1000	NCI, 1979
Rat	3.95	40.5	3.1	Significantly decreased lifespan	10	30	100	NCI, 1979

As seen from Table 9-2, the highest CS and lowest RQ was obtained from the data for increased mortality in female rats in the NCI (1979) study (Table 9-3).

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

Carcinogenicity studies of 2,4-toluenediamine were discussed in Section 5.1. and summarized in Tables 5-1 to 5-7.

The NCI (1979) bioassay of 2,4-toluenediamine demonstrated a carcinogenic effect in female mice (liver tumors), female rats (mammary gland and subcutaneous tumors) and male rats (liver and subcutaneous tumors). At higher levels of dietary contamination, 2,4-toluenediamine resulted in liver (Ito et al., 1969; Weisburger et al., 1978; Du Pont, 1974), lung (Du Pont, 1974) and subcutaneous (Weisburger et al., 1978) tumors in male rats, and in liver (Weisburger et al., 1978; Du Pont, 1974) and mammary gland (Du Pont, 1974) tumors in female rats.

The F-factor for carcinogenicity of 2,4-toluenediamine was calculated from the increased incidence of mammary tumors in female rats (NCI, 1979). Data reported by Du Pont (1974) support the conclusion that the mammary gland is a site of increased tumor formation in rats orally exposed to 2,4-toluenediamine. Data reported by Ito et al. (1969), Weisburger et al. (1978), NCI (1979) and Du Pont (1974) indicate that 2,4-toluenediamine causes an increased incidence of tumors in the livers of rats and mice. Data for the derivation of the F-factor are summarized in Table 9-4. The adjusted $1/ED_{10}$ is $6.48 \text{ (mg/kg/day)}^{-1}$. No unadjusted $1/ED_{10}$ was derived because the corrections for interspecies extrapolation and less than lifetime exposure were applied before using the multistage model.

TABLE 9-3

2,4-Toluenediamine

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	40.5 mg/day
Effect:	significantly decreased lifespan
Reference:	NCI, 1979
RV _d :	3.1
RV _e :	10
Composite Score:	31
RQ:	100

*Equivalent human dose

TABLE 9-4

Derivation of Potency Factor (F) for 2,4-Toluenediamine

Reference:	NCI, 1979
Exposure route:	oral
Species:	rat
Strain:	F344
Sex:	female
Vehicle or physical state:	diet
Body weight:	0.175 kg (high dose), 0.22 kg (low dose), 0.275 kg (control)
Duration of treatment:	103 weeks (control and low dose); 84 weeks (high dose)
Duration of study:	103 weeks (control and low dose); 84 weeks (high dose)
Lifespan of animal:	104 weeks
Target organ:	mammary gland
Tumor type:	adenoma/carcinoma
Experimental doses/ exposure (ppm):	171 (high dose), 79 (low dose), 0 (control)
Transformed dose (mg/kg/day):	0.61 (high dose), 0.56 (low dose), 0 (control)
Tumor incidence:	41/50 (high dose), 38/50 (low dose), 1/20 (control)
Adjusted 1/ED ₁₀ (F factor):	6.48 (mg/kg/day) ⁻¹

In a previous determination (SRC, 1983), the data for increased incidences of hepatocellular carcinoma in female mice were used as the basis of the $1/ED_{10}$ of $2.95 \text{ (mg/kg/day)}^{-1}$. The $1/ED_{10}$ of $6.48 \text{ (mg/kg/day)}^{-1}$ based on mammary tumor data in female rats is clearly a more conservative estimate.

Based on the adequate evidence of carcinogenicity in rats and mice, and on the lack of evidence concerning the carcinogenicity of 2,4-toluenediamine in humans, 2,4-TDA is most appropriately classified as an EPA category B2 chemical which suggests 2,4-toluenediamine is probably carcinogenic to man.

Because the $1/ED_{10}$ falls between 1 and 10, 2,4-toluenediamine is placed in CERCLA Potency Group 3. A Potency Group 3 chemical with an EPA Group B2 (U.S. EPA, 1984) ranks medium in the Hazard Ranking Scheme for Reportable Quantities under CERCLA.

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APPENDIX
LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

CAS online (U.S. EPA Chemical Activities Status Report)
CAS online STN Internation
TOXLINE
TOXBACK 76
TOXBACK 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register

These searches were conducted in August, 1985. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

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